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Colleen Hanagan

NATIONAL STAGE APPLICATION TRANSMITTAL LETTER
APPLICATION FILING UNDER 35 U.S.C. § 371

Transmitted herewith for filing is the patent application of:

Inventor(s)/Applicant(s):	Ruelle, Jean-Louis and Thonnard, Joelle
International Application No.:	PCT/EP00/00135
International Published Appln. No.:	WO 00/42191
International Filing Date:	10 January 2000
Priority Filing Dates:	15 January 1999, 28 January 1999, 29 January 1999, 09 February 1999, 10 February 1999, 20 February 1999, 23 February 1999 & 25 February 1999
Thirty Month Date:	15 July 2001
Title:	"NOVEL COMPOUNDS"

1. **THIS NEW APPLICATION IS A NATIONAL STAGE APPLICATION UNDER PCT, CHAPTER II WITH A REQUEST FOR EXAMINATION WITHOUT DELAY TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US).**

- ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. § 371;
- ☐ This is a **SECOND** or subsequent submission of items concerning a filing under 35 U.S.C. § 371.

2. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

3. A proper Demand for International Preliminary Examination was made by the 19TH month from the earliest claimed priority date.

4. Enclosed items are required for filing under 37 CFR § 1.53(b) and § 1.494(b) or § 1.495(b):

- ☒ One copy of International Publication No. WO 00/42191
- (a) ☒ is transmitted herewith (**required only if not transmitted by the International Bureau**)
- (b) ☐ has been transmitted by the International Bureau
- (c) ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)

Fees

☒ The basic national fee set forth in 37 CFR § 1.482 - International Preliminary Examination Fee not paid to USPTO but International Search Report prepared by the EPO or JPO - **\$860.00**

- ☒ Claims in Excess of 20 (2 @ \$18.00)
- ☐ Independent Claims in Excess of 3 (@ \$80.00)

5. Further enclosed are:

- ☒ One copy of International Preliminary Examination Report.
- ☒ One copy of International Search Report.
- ☐ One copy of Written Opinion.
- ☐ One copy of PCT Request as filed.
- ☐ One copy of Chapter II Demand as filed.

6. ☐ A translation of the International Application into English (35 U.S.C. § 371(c)(2))

7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. § 371(c)(3))

8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. § 371(c)(3))

9. Still additional papers enclosed:

- ☐ Assignment with Assignment Recordation Form Cover Sheet
- ☐ Verification Statement Claiming Small Entity Status
- ☐ Declaration or oath is enclosed executed by the inventor
- ☐ An Information Disclosure Statement under 37 CFR § 1.97 and § 1.98
- ☒ Return Acknowledgment Postcard

10. Nucleotide and/or Amino Acid Sequence Submission (if applicable, all necessary)
- A. Enclosed are:
- (a) ☒ Computer Readable Copy of the Sequence Listing
- (b) ☒ Paper Copy (identical to Computer Readable Copy) of the Sequence Listing
- B. ☐ Enclosed is a paper copy of the Sequence Listing. This paper copy and a Computer Readable Form thereof are identical with the Computer Readable Form in another application of the Applicant which is fully identified as follows:
- U.S. Application No.: @@
- Filed: @@
- Attorney Docket No.: @@
- which is believed to comply with the rules set forth in 37 CFR § 1.821 et. seq. Applicants requests pursuant to 37 CFR § 1.821(e) that this Computer Readable Form be used in the present application. **Please TRANSFER the sequence listing from the parent to this application.**
- C. ☒ Statement under 37 CFR § 1.821(f): **The information recorded in computer readable form is identical to the written Sequence Listing.**
- D. ☐ Statement under 37 CFR § 1.821(g) (required when Sequence Listing not submitted at the time of filing under 35 U.S.C. §111(a)) or 37 CFR §1.821(f) (required when Sequence Listing not submitted at the time of filing under the Patent Cooperation Treaty): **The submission of the Sequence Listing includes no new matter.**
- E. ☒ Amendment: Please enter the Sequence Listing into the application.

11. **Preliminary Amendment**

Prior to calculation of fees, kindly enter:

- ☒ Preliminary Amendment submitted herewith
- ☐ do not enter Preliminary Amendment

09/889283
JC19 Rec'd PCT/PTO
PATENT 1 3 JUL 2001
DOCKET NO. BM45348

12. The correspondence address for this application is the Customer No. provided below:

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PATENT TRADEMARK OFFICE

13. Fee payment being made at this time is enclosed:

* Basic filing fee (\$860.00)	860.00
* Claims in Excess of 20	36.00
* (2 @ \$18.00)	
Independent Claims in Excess of 3	00.00
(@ \$80.00)	
* Total Fees enclosed:	<u>\$896.00</u>

14. The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Account No. 50-0258. This letter is filed in duplicate for accounting purposes.

Respectfully submitted,

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Date: July 13, 2001

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International Application No.: PCT/EP00/00135
Attorney Docket No.: BM45348

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

#7/a

Applicant: Ruelle et al.
Serial No.: Unknown
Filed: Herewith
For: Novel Compounds

Group Art Unit No.: Unknown
Examiner: Unknown

PRELIMINARY AMENDMENT

Sir:

Applicant respectfully requests that this Preliminary Amendment be entered in this case before the calculation of fees and before examination of the subject application.

In the Claims:

Please delete the claims of the application as filed in the PCT and substitute therefor:

25. An isolated polypeptide comprising a member selected from the group consisting of
- (a) an amino acid sequence matching one of SEQ ID NOs: 2 (BASB051), 4 (BASB057), 6 (BASB060), 8 (BASB061), 10 (BASB063), 12 (BASB065), 14 (BASB066), or 16 (BASB071);
 - (b) an immunogenic polypeptide comprising a fragment sequence of at least 15 amino acids that matches an aligned contiguous segment of one of SEQ ID NOs: 4, 6, 8, 10, 12, 14, or 16;

wherein the isolated polypeptide, when administered to a subject in a suitable composition which can include an adjuvant, or a suitable carrier coupled to the polypeptide, induces an antibody or T-cell immune response to a polypeptide having the sequence of one of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, or 16.

26. An isolated polynucleotide encoding a polypeptide of Claim 25 or the full complement to the isolated polynucleotide.

27. The isolated polypeptide of claim 25, wherein the polypeptide is according to (a).

28. An isolated polynucleotide encoding a polypeptide of Claim 27 or the full complement to the isolated polynucleotide.

29. The isolated polypeptide of claim 25, wherein the polypeptide is according to (b).
30. An isolated polynucleotide encoding a polypeptide of Claim 29 or the full complement to the isolated polynucleotide.
31. The isolated polypeptide of claim 25, wherein the immunogenic fragment of (b) comprises at least 20 amino acids.
32. The isolated polypeptide of Claim 25 wherein the isolated polypeptide of (a) consists of one of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, and 16.
33. An isolated polynucleotide encoding a polypeptide of Claim 32 or the full complement to the isolated polynucleotide.
34. A process for expressing the polynucleotide of Claim 33 comprising transforming a host cell with an expression vector comprising the polynucleotide and culturing the host cell under conditions sufficient for expression of the polynucleotide.
35. A fusion protein comprising the isolated polypeptide of Claim 25.
36. An isolated polynucleotide comprising the polynucleotide of one of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, or 15.-
37. An isolated polynucleotide segment comprising a polynucleotide sequence or the full complement of the entire length of the polynucleotide sequence, wherein the polynucleotide sequence hybridizes to the full complement of one of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, or 15 minus the complement of any stop codon, wherein the hybridization conditions include incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 micrograms/ml denatured, sheared salmon sperm DNA, followed by washing in 0.1x SSC at 65°C; and, wherein the polynucleotide sequence is identical to one of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, or 15 minus any terminal stop codon, except that, over the entire length corresponding to one of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, or 15 minus any terminal stop

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codon, n_n nucleotides are substituted, inserted or deleted, wherein n_n satisfies the following expression

$$n_n \leq x_n - (x_n \bullet y)$$

wherein x_n is the total number of nucleotides in SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, or 15 minus any terminal stop codon, y is at least 0.95, and wherein any non-integer product of x_n and y is rounded down to the nearest integer before subtracting the product from x_n ; and wherein the polynucleotide sequence detects *Neisseria meningitidis*.

38. An expression vector comprising the isolated polynucleotide of Claim 26.
39. A host cell transformed with the expression vector of Claim 38.
40. A vaccine comprising the polypeptide of Claim 25 and a pharmaceutically acceptable carrier.
41. The vaccine of Claim 40, wherein the vaccine comprises at least one other *Neisseria meningitidis* antigen.
42. An antibody immunospecific for the polypeptide or immunogenic fragment of Claim 25.
43. A method for inducing an immune response in a mammal comprising administration of the polypeptide of Claim 25.
44. A method of diagnosing a *Neisseria meningitidis* infection, comprising identifying a polypeptide of Claim 25, or an antibody that is immunospecific for the polypeptide, present within a biological sample from an animal suspected of having such an infection.
45. A method for inducing an immune response in a mammal comprising administration of the isolated polynucleotide of Claim 26.

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46. A therapeutic composition useful in treating humans with *Neisseria meningitidis* comprising at least one antibody directed against the polypeptide of claim 25 and a suitable pharmaceutical carrier.

REMARKS

Claims

Claims 1-24 have been canceled without prejudice or disclaimer of the subject matter therein. Applicant reserves the right to prosecute, in one or more patent applications, the canceled claims, the claims as originally filed, and any other claims supported by the specification.

New claims 25-46 have been introduced. No new matter is added.

Support

Support for the new claims is either obvious, or is as described in the text below. Support for compositions of the isolated polypeptide which include an adjuvant recited in the claims may be found, for example, at page 94, lines 15-16. Support for the hybridization conditions may be found, for example, at page 51, lines 27-30. Support for the recitation of sequence relatedness such as in claim 37 may be found in the specification, for example, at page 97, line 21 through page 98, line 15.

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Closing Remarks

Allowance of the pending claims is respectfully requested.

Respectfully submitted,



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Novel Compounds

5

FIELD OF THE INVENTION

This invention relates to polynucleotides, (herein referred to as "BASB051 polynucleotide(s)", "BASB057 polynucleotide(s)", "BASB060 polynucleotide(s)", "BASB061 polynucleotide(s)", "BASB063 polynucleotide(s)", "BASB065 polynucleotide(s)", "BASB066 polynucleotide(s)" and "BASB071 polynucleotide(s)"), polypeptides encoded by them (referred to herein as "BASB051", "BASB057", "BASB060", "BASB061", "BASB063", "BASB065", "BASB066" and "BASB071" respectively or "BASB051 polypeptide(s)", "BASB057 polypeptide(s)", "BASB060 polypeptide(s)", "BASB061 polypeptide(s)", "BASB063 polypeptide(s)", "BASB065 polypeptide(s)", "BASB066 polypeptide(s)" and "BASB071 polypeptide(s)" respectively), recombinant materials and methods for their production. In another aspect, the invention relates to methods for using such polypeptides and polynucleotides, including vaccines against bacterial infections. In a further aspect, the invention relates to diagnostic assays for detecting infection of certain pathogens.

20

BACKGROUND OF THE INVENTION

Neisseria meningitidis (meningococcus) is a Gram-negative bacterium frequently isolated from the human upper respiratory tract. It occasionally causes invasive bacterial diseases such as bacteremia and meningitis. The incidence of meningococcal disease shows geographical seasonal and annual differences (Schwartz, B., Moore, P.S., Broome, C.V.; Clin. Microbiol. Rev. 2 (Supplement), S18-S24, 1989). Most disease in temperate countries is due to strains of serogroup B and varies in incidence from 1-10/100,000/year total population sometimes reaching higher values (Kaczmarek, E.B. (1997), Commun. Dis. Rep. Rev. 7: R55-9, 1995; Scholten, R.J.P.M., Bijlmer, H.A., Poolman, J.T. et al. Clin. Infect. Dis. 16: 237-246, 1993; Cruz, C., Pavez, G., Aguilar, E., et al. Epidemiol. Infect. 105: 119-126, 1990).

30

Inf. Immun. 63: 884-890, 1995). The mechanisms of protective immunity will involve antibody mediated bactericidal activity and opsonophagocytosis.

- 5 A bacteremia animal model has been used to combine all antibody mediated mechanisms (Saukkonen, K., Leinonen, M., Abdillahi, H. Poolman, J. T. Vaccine 7: 325-328, 1989). It is generally accepted that the late complement component mediated bactericidal mechanism is crucial for immunity against meningococcal disease (Ross, S.C., Rosenthal P.J., Berberic, H.M., Densen, P. J. Infect. Dis. 155: 1266-1275, 1987).
- 10 The frequency of *Neisseria meningitidis* infections has risen dramatically in the past few decades. This has been attributed to the emergence of multiply antibiotic resistant strains and an increasing population of people with weakened immune systems. It is no longer uncommon to isolate *Neisseria meningitidis* strains that are resistant to some or all of the standard antibiotics. This phenomenon has created an unmet medical need and demand for
- 15 new anti-microbial agents, vaccines, drug screening methods, and diagnostic tests for this organism.

SUMMARY OF THE INVENTION

- 20 The present invention relates to BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066, and BASB071, in particular BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066, and BASB071 polypeptides and BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066, and BASB071
- 25 polynucleotides, recombinant materials and methods for their production. In another aspect, the invention relates to methods for using such polypeptides and polynucleotides, including prevention and treatment of microbial diseases, amongst others. In a further aspect, the invention relates to diagnostic assays for detecting diseases associated with microbial infections and conditions associated with such infections, such as assays for detecting expression or activity of BASB051, BASB057, BASB060, BASB061, BASB063,
- 30 BASB065, BASB066, and BASB071 polynucleotides or polypeptides.

Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following descriptions and from reading the other parts of the present disclosure.

5

DESCRIPTION OF THE INVENTION

The invention relates to BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066, and BASB071 polypeptides and polynucleotides as described in greater detail
 10 below. The invention relates especially to BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066, and BASB071 having the nucleotide and amino acid sequences set out in SEQ ID NO:1,3,5,7,9,11,13,15 and SEQ ID NO:2,4,6,8,10,12,14,16 respectively. It is understood that sequences recited in the Sequence Listing below as “DNA” represent an exemplification of one embodiment of the invention, since those of
 15 ordinary skill will recognize that such sequences can be usefully employed in polynucleotides in general, including ribopolynucleotides.

Polypeptides

In one aspect of the invention there are provided polypeptides of *Neisseria meningitidis*
 20 referred to herein as “BASB051”, “BASB057”, “BASB060”, “BASB061”, “BASB063”, “BASB065”, “BASB066” and “BASB071”, and “BASB051 polypeptides”, “BASB057 polypeptides”, “BASB060 polypeptides”, “BASB061 polypeptides”, “BASB063 polypeptides”, “BASB065 polypeptides”, “BASB066 polypeptides”, and “BASB071 polypeptides” as well as biologically, diagnostically, prophylactically, clinically or
 25 therapeutically useful variants thereof, and compositions comprising the same.

The present invention further provides for:

(a) an isolated polypeptide which comprises an amino acid sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% or exact identity, to that of SEQ ID NO:2.

(b) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:1 over the entire length of SEQ ID NO:1.

(c) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity, to the amino acid sequence of SEQ ID NO:2.

The BASB051 polypeptide provided in SEQ ID NO:2 is the BASB051 polypeptide from *Neisseria meningitidis* strain ATCC13090.

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The invention also provides an immunogenic fragment of a BASB051 polypeptide, that is, a contiguous portion of the BASB051 polypeptide which has the same or substantially the same immunogenic activity as the polypeptide comprising the amino acid sequence of SEQ ID NO:2. That is to say, the fragment (if necessary when coupled to a carrier) is capable of raising an immune response which recognises the BASB051 polypeptide. Such an immunogenic fragment may include, for example, the BASB051 polypeptide lacking an N-terminal leader sequence, and/or a transmembrane domain and/or a C-terminal anchor domain. In a preferred aspect the immunogenic fragment of BASB051 according to the invention comprises substantially all of the extracellular domain of a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% identity, to that of SEQ ID NO:2 over the entire length of SEQ ID NO:2.

25

The present invention further provides for:

(a) an isolated polypeptide which comprises an amino acid sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% or exact identity, to that of SEQ ID NO:6.

(b) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:5 over the entire length of SEQ ID NO:5.

(c) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity, to the amino acid sequence of SEQ ID NO:6

The BASB060 polypeptide provided in SEQ ID NO:6 is the BASB060 polypeptide from *Neisseria meningitidis* strain ATCC13090.

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The invention also provides an immunogenic fragment of a BASB060 polypeptide, that is, a contiguous portion of the BASB060 polypeptide which has the same or substantially the same immunogenic activity as the polypeptide comprising the amino acid sequence of SEQ ID NO:6. That is to say, the fragment (if necessary when coupled to a carrier) is capable of raising an immune response which recognises the BASB060 polypeptide. Such an immunogenic fragment may include, for example, the BASB060 polypeptide lacking an N-terminal leader sequence, and/or a transmembrane domain and/or a C-terminal anchor domain. In a preferred aspect the immunogenic fragment of BASB060 according to the invention comprises substantially all of the extracellular domain of a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% identity, to that of SEQ ID NO:6 over the entire length of SEQ ID NO:6.

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The present invention further provides for:

(a) an isolated polypeptide which comprises an amino acid sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% or exact identity, to that of SEQ ID NO:10.

(b) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:9 over the entire length of SEQ ID NO:9.

(c) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity, to the amino acid sequence of SEQ ID NO:10

The BASB063 polypeptide provided in SEQ ID NO:10 is the BASB063 polypeptide from *Neisseria meningitidis* strain ATCC13090.

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The invention also provides an immunogenic fragment of a BASB063 polypeptide, that is, a contiguous portion of the BASB063 polypeptide which has the same or substantially the same immunogenic activity as the polypeptide comprising the amino acid sequence of SEQ ID NO:10. That is to say, the fragment (if necessary when coupled to a carrier) is capable of raising an immune response which recognises the BASB063 polypeptide. Such an immunogenic fragment may include, for example, the BASB063 polypeptide lacking an N-terminal leader sequence, and/or a transmembrane domain and/or a C-terminal anchor domain. In a preferred aspect the immunogenic fragment of BASB063 according to the invention comprises substantially all of the extracellular domain of a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% identity, to that of SEQ ID NO:10 over the entire length of SEQ ID NO:10.

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The present invention further provides for:

(a) an isolated polypeptide which comprises an amino acid sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% or exact identity, to that of SEQ ID NO:12.

(b) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:11 over the entire length of SEQ ID NO:11.

(c) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity, to the amino acid sequence of SEQ ID NO:12

The BASB065 polypeptide provided in SEQ ID NO:12 is the BASB065 polypeptide from *Neisseria meningitidis* strain ATCC13090.

The invention also provides an immunogenic fragment of a BASB065 polypeptide, that is, a contiguous portion of the BASB065 polypeptide which has the same or substantially the same immunogenic activity as the polypeptide comprising the amino acid sequence of SEQ ID NO:12. That is to say, the fragment (if necessary when coupled to a carrier) is capable of raising an immune response which recognises the BASB065 polypeptide. Such an immunogenic fragment may include, for example, the BASB065 polypeptide lacking an N-terminal leader sequence, and/or a transmembrane domain and/or a C-terminal anchor domain. In a preferred aspect the immunogenic fragment of BASB065 according to the invention comprises substantially all of the extracellular domain of a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% identity, to that of SEQ ID NO:12 over the entire length of SEQ ID NO:12.

The present invention further provides for:

- (a) an isolated polypeptide which comprises an amino acid sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% or exact identity, to that of SEQ ID NO:14.
- (b) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:13 over the entire length of SEQ ID NO:13.
- (c) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity, to the amino acid sequence of SEQ ID NO:14

The BASB066 polypeptide provided in SEQ ID NO:14 is the BASB066 polypeptide from *Neisseria meningitidis* strain ATCC13090.

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The invention also provides an immunogenic fragment of a BASB066 polypeptide, that is, a contiguous portion of the BASB066 polypeptide which has the same or substantially the same immunogenic activity as the polypeptide comprising the amino acid sequence of SEQ ID NO:14. That is to say, the fragment (if necessary when coupled to a carrier) is capable of raising an immune response which recognises the BASB066 polypeptide. Such an immunogenic fragment may include, for example, the BASB066 polypeptide lacking an N-terminal leader sequence, and/or a transmembrane domain and/or a C-terminal anchor domain. In a preferred aspect the immunogenic fragment of BASB066 according to the invention comprises substantially all of the extracellular domain of a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% identity, to that of SEQ ID NO:14 over the entire length of SEQ ID NO:14.

The present invention further provides for:

The polypeptides, or immunogenic fragments, of the invention may be in the form of the "mature" protein or may be a part of a larger protein such as a precursor or a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in

5 purification such as multiple histidine residues, or an additional sequence for stability during recombinant production. Furthermore, addition of exogenous polypeptide or lipid tail or polynucleotide sequences to increase the immunogenic potential of the final molecule is also considered.

- 10 In one aspect, the invention relates to genetically engineered soluble fusion proteins comprising a polypeptide of the present invention, or a fragment thereof, and various portions of the constant regions of heavy or light chains of immunoglobulins of various subclasses (IgG, IgM, IgA, IgE). Preferred as an immunoglobulin is the constant part of the heavy chain of human IgG, particularly IgG1, where fusion takes place at the hinge
- 15 region. In a particular embodiment, the Fc part can be removed simply by incorporation of a cleavage sequence which can be cleaved with blood clotting factor Xa.

Furthermore, this invention relates to processes for the preparation of these fusion proteins by genetic engineering, and to the use thereof for drug screening, diagnosis and

20 therapy. A further aspect of the invention also relates to polynucleotides encoding such fusion proteins. Examples of fusion protein technology can be found in International Patent Application Nos. WO94/29458 and WO94/22914.

The proteins may be chemically conjugated, or expressed as recombinant fusion proteins

25 allowing increased levels to be produced in an expression system as compared to non-fused protein. The fusion partner may assist in providing T helper epitopes (immunological fusion partner), preferably T helper epitopes recognised by humans, or assist in expressing the protein (expression enhancer) at higher yields than the native recombinant protein. Preferably the fusion partner will be both an immunological fusion

30 partner and expression enhancing partner.

Fusion partners include protein D from *Haemophilus influenzae* and the non-structural protein from influenzae virus, NS1 (hemagglutinin). Another fusion partner is the protein known as LytA. Preferably the C terminal portion of the molecule is used. LytA is
5 derived from *Streptococcus pneumoniae* which synthesize an N-acetyl-L-alanine amidase, amidase LytA, (coded by the lytA gene {Gene, 43 (1986) page 265-272}) an autolysin that specifically degrades certain bonds in the peptidoglycan backbone. The C-terminal domain of the LytA protein is responsible for the affinity to the choline or to some choline analogues such as DEAE. This property has been exploited for the
10 development of *E.coli* C-LytA expressing plasmids useful for expression of fusion proteins. Purification of hybrid proteins containing the C-LytA fragment at its amino terminus has been described {Biotechnology: 10, (1992) page 795-798}. It is possible to use the repeat portion of the LytA molecule found in the C terminal end starting at residue 178, for example residues 188 - 305.

15 The present invention also includes variants of the aforementioned polypeptides, that is polypeptides that vary from the referents by conservative amino acid substitutions, whereby a residue is substituted by another with like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu;
20 among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr.

Polypeptides of the present invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced
25 polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

It is most preferred that a polypeptide of the invention is derived from *Neisseria meningitidis*,
30 however, it may preferably be obtained from other organisms of the same taxonomic genus.

A polypeptide of the invention may also be obtained, for example, from organisms of the same taxonomic family or order.

Polynucleotides

- 5 It is an object of the invention to provide polynucleotides that encode BASB051 polypeptides, particularly polynucleotides that encode the polypeptide herein designated BASB051.

- 10 In a particularly preferred embodiment of the invention the polynucleotide comprises a region encoding BASB051 polypeptides comprising a sequence set out in SEQ ID NO:1 which includes a full length gene, or a variant thereof.

The BASB051 polynucleotide provided in SEQ ID NO:1 is the BASB051 polynucleotide from *Neisseria meningitidis* strains ATCC13090.

15

- As a further aspect of the invention there are provided isolated nucleic acid molecules encoding and/or expressing BASB051 polypeptides and polynucleotides, particularly *Neisseria meningitidis* BASB051 polypeptides and polynucleotides, including, for example, unprocessed RNAs, ribozyme RNAs, mRNAs, cDNAs, genomic DNAs, B- and
20 Z-DNAs. Further embodiments of the invention include biologically, diagnostically, prophylactically, clinically or therapeutically useful polynucleotides and polypeptides, and variants thereof, and compositions comprising the same.

- Another aspect of the invention relates to isolated polynucleotides, including at least one full
25 length gene, that encodes a BASB051 polypeptide having a deduced amino acid sequence of SEQ ID NO:2 and polynucleotides closely related thereto and variants thereof.

- In another particularly preferred embodiment of the invention there is a BASB051 polypeptide from *Neisseria meningitidis* comprising or consisting of an amino acid
30 sequence of SEQ ID NO:2 or a variant thereof.

Using the information provided herein, such as a polynucleotide sequence set out in SEQ ID NO:1 a polynucleotide of the invention encoding BASB051 polypeptide may be obtained using standard cloning and screening methods, such as those for cloning and sequencing chromosomal DNA fragments from bacteria using *Neisseria meningitidis* cells as starting material, followed by obtaining a full length clone. For example, to obtain a polynucleotide sequence of the invention, such as a polynucleotide sequence given in SEQ ID NO:1 typically a library of clones of chromosomal DNA of *Neisseria meningitidis* in *E. coli* or some other suitable host is probed with a radiolabeled oligonucleotide, preferably a 17-mer or longer, derived from a partial sequence. Clones carrying DNA identical to that of the probe can then be distinguished using stringent hybridization conditions. By sequencing the individual clones thus identified by hybridization with sequencing primers designed from the original polypeptide or polynucleotide sequence it is then possible to extend the polynucleotide sequence in both directions to determine a full length gene sequence. Conveniently, such sequencing is performed, for example, using denatured double stranded DNA prepared from a plasmid clone. Suitable techniques are described by Maniatis, T., Fritsch, E.F. and Sambrook et al., *MOLECULAR CLONING, A LABORATORY MANUAL*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989). (see in particular Screening By Hybridization 1.90 and Sequencing Denatured Double-Stranded DNA Templates 13.70). Direct genomic DNA sequencing may also be performed to obtain a full length gene sequence. Illustrative of the invention, each polynucleotide set out in SEQ ID NO:1 was discovered in a DNA library derived from *Neisseria meningitidis*.

Moreover, the DNA sequence set out in SEQ ID NO:1 contains an open reading frame
25 encoding a protein having about the number of amino acid residues set forth in SEQ ID NO:2
with a deduced molecular weight that can be calculated using amino acid residue molecular
weight values well known to those skilled in the art.

hybridization occurring only if there is at least 95% and preferably at least 97% identity between the sequences. A specific example of stringent hybridization conditions is overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 5 10% dextran sulfate, and 20 micrograms/ml of denatured, sheared salmon sperm DNA, followed by washing the hybridization support in 0.1x SSC at about 65°C. Hybridization and wash conditions are well known and exemplified in Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), particularly Chapter 11 therein. Solution hybridization may also be used with the 10 polynucleotide sequences provided by the invention.

The invention also provides a polynucleotide consisting of or comprising a polynucleotide sequence obtained by screening an appropriate library containing the complete gene for a polynucleotide sequence set forth in SEQ ID NO:1 under stringent hybridization conditions 15 with a probe having the sequence of said polynucleotide sequence set forth in SEQ ID NO:1 or a fragment thereof; and isolating said polynucleotide sequence. Fragments useful for obtaining such a polynucleotide include, for example, probes and primers fully described elsewhere herein.

20 As discussed elsewhere herein regarding polynucleotide assays of the invention, for instance, the polynucleotides of the invention, may be used as a hybridization probe for RNA, cDNA and genomic DNA to isolate full-length cDNAs and genomic clones encoding BASB051 and to isolate cDNA and genomic clones of other genes that have a high identity, particularly high sequence identity, to the BASB051 gene. Such probes generally will comprise at least 15 25 nucleotide residues or base pairs. Preferably, such probes will have at least 30 nucleotide residues or base pairs and may have at least 50 nucleotide residues or base pairs. Particularly preferred probes will have at least 20 nucleotide residues or base pairs and will have less than 30 nucleotide residues or base pairs.

A coding region of a BASB051 gene may be isolated by screening using a DNA sequence provided in SEQ ID NO:1 to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the invention is then used to screen a library of cDNA, genomic DNA or mRNA to determine which members of the library the
5 probe hybridizes to.

It is an object of the invention to provide polynucleotides that encode BASB057 polypeptides, particularly polynucleotides that encode the polypeptide herein designated BASB057.

- 5 In a particularly preferred embodiment of the invention the polynucleotide comprises a region encoding BASB057 polypeptides comprising a sequence set out in SEQ ID NO:3 which includes a full length gene, or a variant thereof.

The BASB057 polynucleotide provided in SEQ ID NO:3 is the BASB057 polynucleotide
10 from *Neisseria meningitidis* strains ATCC13090.

As a further aspect of the invention there are provided isolated nucleic acid molecules encoding and/or expressing BASB057 polypeptides and polynucleotides, particularly *Neisseria meningitidis* BASB057 polypeptides and polynucleotides, including, for
15 example, unprocessed RNAs, ribozyme RNAs, mRNAs, cDNAs, genomic DNAs, B- and Z-DNAs. Further embodiments of the invention include biologically, diagnostically, prophylactically, clinically or therapeutically useful polynucleotides and polypeptides, and variants thereof, and compositions comprising the same.

- 20 Another aspect of the invention relates to isolated polynucleotides, including at least one full length gene, that encodes a BASB057 polypeptide having a deduced amino acid sequence of SEQ ID NO:4 and polynucleotides closely related thereto and variants thereof.

In another particularly preferred embodiment of the invention there is a BASB057
25 polypeptide from *Neisseria meningitidis* comprising or consisting of an amino acid sequence of SEQ ID NO:4 or a variant thereof.

Using the information provided herein, such as a polynucleotide sequence set out in SEQ ID NO:3 a polynucleotide of the invention encoding BASB057 polypeptide may be obtained
30 using standard cloning and screening methods, such as those for cloning and sequencing

chromosomal DNA fragments from bacteria using *Neisseria meningitidis* cells as starting material, followed by obtaining a full length clone. For example, to obtain a polynucleotide sequence of the invention, such as a polynucleotide sequence given in SEQ ID NO:3

typically a library of clones of chromosomal DNA of *Neisseria meningitidis* in *E.coli* or

5 some other suitable host is probed with a radiolabeled oligonucleotide, preferably a 17-mer or longer, derived from a partial sequence. Clones carrying DNA identical to that of the

probe can then be distinguished using stringent hybridization conditions. By sequencing the individual clones thus identified by hybridization with sequencing primers designed

from the original polypeptide or polynucleotide sequence it is then possible to extend the

10 polynucleotide sequence in both directions to determine a full length gene sequence.

Conveniently, such sequencing is performed, for example, using denatured double stranded DNA prepared from a plasmid clone. Suitable techniques are described by Maniatis, T.,

Fritsch, E.F. and Sambrook et al., *MOLECULAR CLONING, A LABORATORY MANUAL*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989). (see

15 in particular Screening By Hybridization 1.90 and Sequencing Denatured Double-Stranded DNA Templates 13.70). Direct genomic DNA sequencing may also be performed to obtain

a full length gene sequence. Illustrative of the invention, each polynucleotide set out in SEQ ID NO:3 was discovered in a DNA library derived from *Neisseria meningitidis*.

20 Moreover, the DNA sequence set out in SEQ ID NO:3 contains an open reading frame encoding a protein having about the number of amino acid residues set forth in SEQ ID NO:4 with a deduced molecular weight that can be calculated using amino acid residue molecular weight values well known to those skilled in the art.

25 The polynucleotide of SEQ ID NO:3, between the start codon at nucleotide number 1 and the stop codon which begins at nucleotide number 1402 of SEQ ID NO:3, encodes the polypeptide of SEQ ID NO:4.

In a further aspect, the present invention provides for an isolated polynucleotide comprising
30 or consisting of:

- (a) a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:3 over the entire length of SEQ ID NO:3; or
- (b) a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or 100% exact, to the amino acid sequence of SEQ ID NO:4 over the entire length of SEQ ID NO:4.

A polynucleotide encoding a polypeptide of the present invention, including homologs and orthologs from species other than *Neisseria meningitidis*, may be obtained by a process which comprises the steps of screening an appropriate library under stringent hybridization conditions (for example, using a temperature in the range of 45 – 65°C and an SDS concentration from 0.1 – 1%) with a labeled or detectable probe consisting of or comprising the sequence of SEQ ID NO:3 or a fragment thereof; and isolating a full-length gene and/or genomic clones containing said polynucleotide sequence.

The invention provides a polynucleotide sequence identical over its entire length to a coding sequence (open reading frame) in SEQ ID NO:3. Also provided by the invention is a coding sequence for a mature polypeptide or a fragment thereof, by itself as well as a coding sequence for a mature polypeptide or a fragment in reading frame with another coding sequence, such as a sequence encoding a leader or secretory sequence, a pre-, or pro- or prepro-protein sequence. The polynucleotide of the invention may also contain at least one non-coding sequence, including for example, but not limited to at least one non-coding 5' and 3' sequence, such as the transcribed but non-translated sequences, termination signals (such as rho-dependent and rho-independent termination signals), ribosome binding sites, Kozak sequences, sequences that stabilize mRNA, introns, and polyadenylation signals. The polynucleotide sequence may also comprise additional coding sequence encoding additional amino acids. For example, a marker sequence that facilitates purification of the fused polypeptide can be encoded. In certain embodiments of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz

et al., *Proc. Natl. Acad. Sci., USA* 86: 821-824 (1989), or an HA peptide tag (*Wilson et al.*, *Cell* 37: 767 (1984), both of which may be useful in purifying polypeptide sequence fused to them. Polynucleotides of the invention also include, but are not limited to, polynucleotides comprising a structural gene and its naturally associated sequences that control gene
5 expression.

The nucleotide sequence encoding BASB057 polypeptide of SEQ ID NO:4 may be identical to the polypeptide encoding sequence contained in nucleotides 1 to 1401 of SEQ ID NO:3. Alternatively it may be a sequence, which as a result of the redundancy
10 (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:4. The term "polynucleotide encoding a polypeptide" as used herein encompasses polynucleotides that include a sequence encoding a polypeptide of the invention, particularly a bacterial polypeptide and more particularly a polypeptide of the *Neisseria meningitidis* BASB057 having an amino acid sequence set out in SEQ ID NO:4. The term also
15 encompasses polynucleotides that include a single continuous region or discontinuous regions encoding the polypeptide (for example, polynucleotides interrupted by integrated phage, an integrated insertion sequence, an integrated vector sequence, an integrated transposon sequence, or due to RNA editing or genomic DNA reorganization) together with additional regions, that also may contain coding and/or non-coding sequences.

20

The invention further relates to variants of the polynucleotides described herein that encode variants of a polypeptide having a deduced amino acid sequence of SEQ ID NO:4.

Fragments of polynucleotides of the invention may be used, for example, to synthesize full-length polynucleotides of the invention.

25

Further particularly preferred embodiments are polynucleotides encoding BASB057 variants, that have the amino acid sequence of BASB057 polypeptide of SEQ ID NO:4 in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, modified, deleted and/or added, in any combination. Especially preferred among these are silent

substitutions, additions and deletions, that do not alter the properties and activities of BASB057 polypeptide.

Further preferred embodiments of the invention are polynucleotides that are at least 85% identical over their entire length to a polynucleotide encoding BASB057 polypeptide having an amino acid sequence set out in SEQ ID NO:4 and polynucleotides that are complementary to such polynucleotides. In this regard, polynucleotides at least 90% identical over their entire length to the same are particularly preferred, and among these particularly preferred polynucleotides, those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred among those with at least 95%, and among these those with at least 98% and at least 99% are particularly highly preferred, with at least 99% being the more preferred.

Preferred embodiments are polynucleotides encoding polypeptides that retain substantially the same biological function or activity as the mature polypeptide encoded by a DNA of SEQ ID NO:3.

In accordance with certain preferred embodiments of this invention there are provided polynucleotides that hybridize, particularly under stringent conditions, to BASB057 polynucleotide sequences, such as those polynucleotides in SEQ ID NO:3.

The invention further relates to polynucleotides that hybridize to the polynucleotide sequences provided herein. In this regard, the invention especially relates to polynucleotides that hybridize under stringent conditions to the polynucleotides described herein. As herein used, the terms "stringent conditions" and "stringent hybridization conditions" mean hybridization occurring only if there is at least 95% and preferably at least 97% identity between the sequences. A specific example of stringent hybridization conditions is overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 micrograms/ml of denatured, sheared salmon sperm DNA,

It is an object of the invention to provide polynucleotides that encode BASB060 polypeptides, particularly polynucleotides that encode the polypeptide herein designated BASB060.

- 5 In a particularly preferred embodiment of the invention the polynucleotide comprises a region encoding BASB060 polypeptides comprising a sequence set out in SEQ ID NO:5 which includes a full length gene, or a variant thereof.

The BASB060 polynucleotide provided in SEQ ID NO:5 is the BASB060 polynucleotide
10 from *Neisseria meningitidis* strains ATCC13090.

As a further aspect of the invention there are provided isolated nucleic acid molecules encoding and/or expressing BASB060 polypeptides and polynucleotides, particularly *Neisseria meningitidis* BASB060 polypeptides and polynucleotides, including, for
15 example, unprocessed RNAs, ribozyme RNAs, mRNAs, cDNAs, genomic DNAs, B- and Z-DNAs. Further embodiments of the invention include biologically, diagnostically, prophylactically, clinically or therapeutically useful polynucleotides and polypeptides, and variants thereof, and compositions comprising the same.

20 Another aspect of the invention relates to isolated polynucleotides, including at least one full length gene, that encodes a BASB060 polypeptide having a deduced amino acid sequence of SEQ ID NO:6 and polynucleotides closely related thereto and variants thereof.

In another particularly preferred embodiment of the invention there is a BASB060
25 polypeptide from *Neisseria meningitidis* comprising or consisting of an amino acid sequence of SEQ ID NO:6 or a variant thereof.

Using the information provided herein, such as a polynucleotide sequence set out in SEQ ID NO:5 a polynucleotide of the invention encoding BASB060 polypeptide may be obtained
30 using standard cloning and screening methods, such as those for cloning and sequencing

chromosomal DNA fragments from bacteria using *Neisseria meningitidis* cells as starting material, followed by obtaining a full length clone. For example, to obtain a polynucleotide sequence of the invention, such as a polynucleotide sequence given in SEQ ID NO:5

typically a library of clones of chromosomal DNA of *Neisseria meningitidis* in *E.coli* or

5 some other suitable host is probed with a radiolabeled oligonucleotide, preferably a 17-mer or longer, derived from a partial sequence. Clones carrying DNA identical to that of the probe can then be distinguished using stringent hybridization conditions. By sequencing the individual clones thus identified by hybridization with sequencing primers designed from the original polypeptide or polynucleotide sequence it is then possible to extend the
10 polynucleotide sequence in both directions to determine a full length gene sequence.

Conveniently, such sequencing is performed, for example, using denatured double stranded DNA prepared from a plasmid clone. Suitable techniques are described by Maniatis, T., Fritsch, E.F. and Sambrook et al., *MOLECULAR CLONING, A LABORATORY MANUAL*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989). (see

15 in particular Screening By Hybridization 1.90 and Sequencing Denatured Double-Stranded DNA Templates 13.70). Direct genomic DNA sequencing may also be performed to obtain a full length gene sequence. Illustrative of the invention, each polynucleotide set out in SEQ ID NO:5 was discovered in a DNA library derived from *Neisseria meningitidis*.

20 Moreover, the DNA sequence set out in SEQ ID NO:5 contains an open reading frame encoding a protein having about the number of amino acid residues set forth in SEQ ID NO:6 with a deduced molecular weight that can be calculated using amino acid residue molecular weight values well known to those skilled in the art.

25 The polynucleotide of SEQ ID NO:5, between the start codon at nucleotide number 1 and the stop codon which begins at nucleotide number 418 of SEQ ID NO:5, encodes the polypeptide of SEQ ID NO:6.

In a further aspect, the present invention provides for an isolated polynucleotide comprising
30 or consisting of:

- (a) a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:5 over the entire length of SEQ ID NO:5; or
- (b) a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or 100% exact, to the amino acid sequence of SEQ ID NO:6 over the entire length of SEQ ID NO:6.

A polynucleotide encoding a polypeptide of the present invention, including homologs and orthologs from species other than *Neisseria meningitidis*, may be obtained by a process which comprises the steps of screening an appropriate library under stringent hybridization conditions (for example, using a temperature in the range of 45 – 65°C and an SDS concentration from 0.1 – 1%) with a labeled or detectable probe consisting of or comprising the sequence of SEQ ID NO:5 or a fragment thereof; and isolating a full-length gene and/or genomic clones containing said polynucleotide sequence.

The invention provides a polynucleotide sequence identical over its entire length to a coding sequence (open reading frame) in SEQ ID NO:5. Also provided by the invention is a coding sequence for a mature polypeptide or a fragment thereof, by itself as well as a coding sequence for a mature polypeptide or a fragment in reading frame with another coding sequence, such as a sequence encoding a leader or secretory sequence, a pre-, or pro- or prepro-protein sequence. The polynucleotide of the invention may also contain at least one non-coding sequence, including for example, but not limited to at least one non-coding 5' and 3' sequence, such as the transcribed but non-translated sequences, termination signals (such as rho-dependent and rho-independent termination signals), ribosome binding sites, Kozak sequences, sequences that stabilize mRNA, introns, and polyadenylation signals. The polynucleotide sequence may also comprise additional coding sequence encoding additional amino acids. For example, a marker sequence that facilitates purification of the fused polypeptide can be encoded. In certain embodiments of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gantz

et al., *Proc. Natl. Acad. Sci., USA* 86: 821-824 (1989), or an HA peptide tag (Wilson *et al.*, *Cell* 37: 767 (1984), both of which may be useful in purifying polypeptide sequence fused to them. Polynucleotides of the invention also include, but are not limited to, polynucleotides comprising a structural gene and its naturally associated sequences that control gene
5 expression.

The nucleotide sequence encoding BASB060 polypeptide of SEQ ID NO:6 may be identical to the polypeptide encoding sequence contained in nucleotides 1 to 417 of SEQ ID NO:5. Alternatively it may be a sequence, which as a result of the redundancy
10 (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:6.

The term "polynucleotide encoding a polypeptide" as used herein encompasses polynucleotides that include a sequence encoding a polypeptide of the invention, particularly a bacterial polypeptide and more particularly a polypeptide of the *Neisseria meningitidis* BASB060 having an amino acid sequence set out in SEQ ID NO:6. The term also
15 encompasses polynucleotides that include a single continuous region or discontinuous regions encoding the polypeptide (for example, polynucleotides interrupted by integrated phage, an integrated insertion sequence, an integrated vector sequence, an integrated transposon sequence, or due to RNA editing or genomic DNA reorganization) together with additional regions, that also may contain coding and/or non-coding sequences.

20 The invention further relates to variants of the polynucleotides described herein that encode variants of a polypeptide having a deduced amino acid sequence of SEQ ID NO:6.

Fragments of polynucleotides of the invention may be used, for example, to synthesize full-length polynucleotides of the invention.

25 Further particularly preferred embodiments are polynucleotides encoding BASB060 variants, that have the amino acid sequence of BASB060 polypeptide of SEQ ID NO:6 in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, modified, deleted and/or added, in any combination. Especially preferred among these are silent

substitutions, additions and deletions that do not alter the properties and activities of BASB060 polypeptide.

Further preferred embodiments of the invention are polynucleotides that are at least 85% identical over their entire length to a polynucleotide encoding BASB060 polypeptide having an amino acid sequence set out in SEQ ID NO:6 and polynucleotides that are complementary to such polynucleotides. In this regard, polynucleotides at least 90% identical over their entire length to the same are particularly preferred, and among these particularly preferred polynucleotides, those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred among those with at least 95%, and among these those with at least 98% and at least 99% are particularly highly preferred, with at least 99% being the more preferred.

Preferred embodiments are polynucleotides encoding polypeptides that retain substantially the same biological function or activity as the mature polypeptide encoded by a DNA of SEQ ID NO:5.

In accordance with certain preferred embodiments of this invention there are provided polynucleotides that hybridize, particularly under stringent conditions, to BASB060 polynucleotide sequences, such as those polynucleotides in SEQ ID NO:5.

The invention further relates to polynucleotides that hybridize to the polynucleotide sequences provided herein. In this regard, the invention especially relates to polynucleotides that hybridize under stringent conditions to the polynucleotides described herein. As herein used, the terms "stringent conditions" and "stringent hybridization conditions" mean hybridization occurring only if there is at least 95% and preferably at least 97% identity between the sequences. A specific example of stringent hybridization conditions is overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 micrograms/ml of denatured, sheared salmon sperm DNA,

followed by washing the hybridization support in 0.1x SSC at about 65°C. Hybridization and wash conditions are well known and exemplified in Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), particularly Chapter 11 therein. Solution hybridization may also be used with the

5 polynucleotide sequences provided by the invention.

The invention also provides a polynucleotide consisting of or comprising a polynucleotide sequence obtained by screening an appropriate library containing the complete gene for a polynucleotide sequence set forth in SEQ ID NO:5 under stringent hybridization conditions

10 with a probe having the sequence of said polynucleotide sequence set forth in SEQ ID NO:5 or a fragment thereof; and isolating said polynucleotide sequence. Fragments useful for obtaining such a polynucleotide include, for example, probes and primers fully described elsewhere herein.

15 As discussed elsewhere herein regarding polynucleotide assays of the invention, for instance, the polynucleotides of the invention, may be used as a hybridization probe for RNA, cDNA and genomic DNA to isolate full-length cDNAs and genomic clones encoding BASB060 and to isolate cDNA and genomic clones of other genes that have a high identity, particularly high sequence identity, to the BASB060 gene. Such probes generally will comprise at least 15

20 nucleotide residues or base pairs. Preferably, such probes will have at least 30 nucleotide residues or base pairs and may have at least 50 nucleotide residues or base pairs. Particularly preferred probes will have at least 20 nucleotide residues or base pairs and will have less than 30 nucleotide residues or base pairs.

25 A coding region of a BASB060 gene may be isolated by screening using a DNA sequence provided in SEQ ID NO:5 to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the invention is then used to screen a library of cDNA, genomic DNA or mRNA to determine which members of the library the probe hybridizes to.

- (a) a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:7 over the entire length of SEQ ID NO:7; or
- (b) a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or 100% exact, to the amino acid sequence of SEQ ID NO:8 over the entire length of SEQ ID NO:8.

A polynucleotide encoding a polypeptide of the present invention, including homologs and orthologs from species other than *Neisseria meningitidis*, may be obtained by a process which comprises the steps of screening an appropriate library under stringent hybridization conditions (for example, using a temperature in the range of 45 – 65°C and an SDS concentration from 0.1 – 1%) with a labeled or detectable probe consisting of or comprising the sequence of SEQ ID NO:7 or a fragment thereof; and isolating a full-length gene and/or genomic clones containing said polynucleotide sequence.

The invention provides a polynucleotide sequence identical over its entire length to a coding sequence (open reading frame) in SEQ ID NO:7. Also provided by the invention is a coding sequence for a mature polypeptide or a fragment thereof, by itself as well as a coding sequence for a mature polypeptide or a fragment in reading frame with another coding sequence, such as a sequence encoding a leader or secretory sequence, a pre-, or pro- or prepro-protein sequence. The polynucleotide of the invention may also contain at least one non-coding sequence, including for example, but not limited to at least one non-coding 5' and 3' sequence, such as the transcribed but non-translated sequences, termination signals (such as rho-dependent and rho-independent termination signals), ribosome binding sites, Kozak sequences, sequences that stabilize mRNA, introns, and polyadenylation signals. The polynucleotide sequence may also comprise additional coding sequence encoding additional amino acids. For example, a marker sequence that facilitates purification of the fused polypeptide can be encoded. In certain embodiments of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz

et al., *Proc. Natl. Acad. Sci., USA* 86: 821-824 (1989), or an HA peptide tag (Wilson *et al.*, *Cell* 37: 767 (1984), both of which may be useful in purifying polypeptide sequence fused to them. Polynucleotides of the invention also include, but are not limited to, polynucleotides comprising a structural gene and its naturally associated sequences that control gene expression.

The nucleotide sequence encoding BASB061 polypeptide of SEQ ID NO:8 may be identical to the polypeptide encoding sequence contained in nucleotides 1 to 513 of SEQ ID NO:7. Alternatively it may be a sequence, which as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:8.

The term "polynucleotide encoding a polypeptide" as used herein encompasses polynucleotides that include a sequence encoding a polypeptide of the invention, particularly a bacterial polypeptide and more particularly a polypeptide of the *Neisseria meningitidis* BASB061 having an amino acid sequence set out in SEQ ID NO:8. The term also encompasses polynucleotides that include a single continuous region or discontinuous regions encoding the polypeptide (for example, polynucleotides interrupted by integrated phage, an integrated insertion sequence, an integrated vector sequence, an integrated transposon sequence, or due to RNA editing or genomic DNA reorganization) together with additional regions, that also may contain coding and/or non-coding sequences.

The invention further relates to variants of the polynucleotides described herein that encode variants of a polypeptide having a deduced amino acid sequence of SEQ ID NO:8.

Fragments of polynucleotides of the invention may be used, for example, to synthesize full-length polynucleotides of the invention.

Further particularly preferred embodiments are polynucleotides encoding BASB061 variants, that have the amino acid sequence of BASB061 polypeptide of SEQ ID NO:8 in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, modified, deleted and/or added, in any combination. Especially preferred among these are silent

substitutions, additions and deletions that do not alter the properties and activities of BASB061 polypeptide.

Further preferred embodiments of the invention are polynucleotides that are at least 85% identical over their entire length to a polynucleotide encoding BASB061 polypeptide having an amino acid sequence set out in SEQ ID NO:8 and polynucleotides that are complementary to such polynucleotides. In this regard, polynucleotides at least 90% identical over their entire length to the same are particularly preferred, and among these particularly preferred polynucleotides, those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred among those with at least 95%, and among these those with at least 98% and at least 99% are particularly highly preferred, with at least 99% being the more preferred.

Preferred embodiments are polynucleotides encoding polypeptides that retain substantially the same biological function or activity as the mature polypeptide encoded by a DNA of SEQ ID NO:7.

In accordance with certain preferred embodiments of this invention there are provided polynucleotides that hybridize, particularly under stringent conditions, to BASB061 polynucleotide sequences, such as those polynucleotides in SEQ ID NO:7.

The invention further relates to polynucleotides that hybridize to the polynucleotide sequences provided herein. In this regard, the invention especially relates to polynucleotides that hybridize under stringent conditions to the polynucleotides described herein. As herein used, the terms "stringent conditions" and "stringent hybridization conditions" mean hybridization occurring only if there is at least 95% and preferably at least 97% identity between the sequences. A specific example of stringent hybridization conditions is overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 micrograms/ml of denatured, sheared salmon sperm DNA,

followed by washing the hybridization support in 0.1x SSC at about 65°C. Hybridization and wash conditions are well known and exemplified in Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), particularly Chapter 11 therein. Solution hybridization may also be used with the
5 polynucleotide sequences provided by the invention.

The invention also provides a polynucleotide consisting of or comprising a polynucleotide sequence obtained by screening an appropriate library containing the complete gene for a polynucleotide sequence set forth in SEQ ID NO:7 under stringent hybridization conditions
10 with a probe having the sequence of said polynucleotide sequence set forth in SEQ ID NO:7 or a fragment thereof; and isolating said polynucleotide sequence. Fragments useful for obtaining such a polynucleotide include, for example, probes and primers fully described elsewhere herein.

As discussed elsewhere herein regarding polynucleotide assays of the invention, for instance, the polynucleotides of the invention, may be used as a hybridization probe for RNA, cDNA and genomic DNA to isolate full-length cDNAs and genomic clones encoding BASB061 and to isolate cDNA and genomic clones of other genes that have a high identity, particularly high sequence identity, to the BASB061 gene. Such probes generally will comprise at least 15
15 nucleotide residues or base pairs. Preferably, such probes will have at least 30 nucleotide residues or base pairs and may have at least 50 nucleotide residues or base pairs. Particularly preferred probes will have at least 20 nucleotide residues or base pairs and will have less than 30 nucleotide residues or base pairs.

A coding region of a BASB061 gene may be isolated by screening using a DNA sequence provided in SEQ ID NO:7 to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the invention is then used to screen a library of cDNA, genomic DNA or mRNA to determine which members of the library the probe hybridizes to.
25

It is an object of the invention to provide polynucleotides that encode BASB063 polypeptides, particularly polynucleotides that encode the polypeptide herein designated BASB063.

- 5 In a particularly preferred embodiment of the invention the polynucleotide comprises a region encoding BASB063 polypeptides comprising a sequence set out in SEQ ID NO:9 which includes a full length gene, or a variant thereof.

- 10 The BASB063 polynucleotide provided in SEQ ID NO:9 is the BASB063 polynucleotide from *Neisseria meningitidis* strains ATCC13090.

- 15 As a further aspect of the invention there are provided isolated nucleic acid molecules encoding and/or expressing BASB063 polypeptides and polynucleotides, particularly *Neisseria meningitidis* BASB063 polypeptides and polynucleotides, including, for example, unprocessed RNAs, ribozyme RNAs, mRNAs, cDNAs, genomic DNAs, B- and Z-DNAs. Further embodiments of the invention include biologically, diagnostically, prophylactically, clinically or therapeutically useful polynucleotides and polypeptides, and variants thereof, and compositions comprising the same.
- 20 Another aspect of the invention relates to isolated polynucleotides, including at least one full length gene, that encodes a BASB063 polypeptide having a deduced amino acid sequence of SEQ ID NO:10 and polynucleotides closely related thereto and variants thereof.

- 25 In another particularly preferred embodiment of the invention there is a BASB063 polypeptide from *Neisseria meningitidis* comprising or consisting of an amino acid sequence of SEQ ID NO:10 or a variant thereof.

- 30 Using the information provided herein, such as a polynucleotide sequence set out in SEQ ID NO:9 a polynucleotide of the invention encoding BASB063 polypeptide may be obtained using standard cloning and screening methods, such as those for cloning and sequencing

chromosomal DNA fragments from bacteria using *Neisseria meningitidis* cells as starting material, followed by obtaining a full length clone. For example, to obtain a polynucleotide sequence of the invention, such as a polynucleotide sequence given in SEQ ID NO:9 typically a library of clones of chromosomal DNA of *Neisseria meningitidis* in *E.coli* or
5 some other suitable host is probed with a radiolabeled oligonucleotide, preferably a 17-mer or longer, derived from a partial sequence. Clones carrying DNA identical to that of the probe can then be distinguished using stringent hybridization conditions. By sequencing the individual clones thus identified by hybridization with sequencing primers designed from the original polypeptide or polynucleotide sequence it is then possible to extend the
10 polynucleotide sequence in both directions to determine a full length gene sequence. Conveniently, such sequencing is performed, for example, using denatured double stranded DNA prepared from a plasmid clone. Suitable techniques are described by Maniatis, T., Fritsch, E.F. and Sambrook et al., *MOLECULAR CLONING, A LABORATORY MANUAL*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989). (see
15 in particular Screening By Hybridization 1.90 and Sequencing Denatured Double-Stranded DNA Templates 13.70). Direct genomic DNA sequencing may also be performed to obtain a full length gene sequence. Illustrative of the invention, each polynucleotide set out in SEQ ID NO:9 was discovered in a DNA library derived from *Neisseria meningitidis*.

20 Moreover, the DNA sequence set out in SEQ ID NO:9 contains an open reading frame encoding a protein having about the number of amino acid residues set forth in SEQ ID NO:10 with a deduced molecular weight that can be calculated using amino acid residue molecular weight values well known to those skilled in the art.

25 The polynucleotide of SEQ ID NO:9, between the start codon at nucleotide number 1 and the stop codon which begins at nucleotide number 814 of SEQ ID NO:9, encodes the polypeptide of SEQ ID NO:10.

In a further aspect, the present invention provides for an isolated polynucleotide comprising
30 or consisting of:

- (a) a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:9 over the entire length of SEQ ID NO:9; or
- (b) a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or 100% exact, to the amino acid sequence of SEQ ID NO:10 over the entire length of SEQ ID NO:10.

A polynucleotide encoding a polypeptide of the present invention, including homologs and orthologs from species other than *Neisseria meningitidis*, may be obtained by a process which comprises the steps of screening an appropriate library under stringent hybridization conditions (for example, using a temperature in the range of 45 – 65°C and an SDS concentration from 0.1 – 1%) with a labeled or detectable probe consisting of or comprising the sequence of SEQ ID NO:9 or a fragment thereof; and isolating a full-length gene and/or genomic clones containing said polynucleotide sequence.

The invention provides a polynucleotide sequence identical over its entire length to a coding sequence (open reading frame) in SEQ ID NO:9. Also provided by the invention is a coding sequence for a mature polypeptide or a fragment thereof, by itself as well as a coding sequence for a mature polypeptide or a fragment in reading frame with another coding sequence, such as a sequence encoding a leader or secretory sequence, a pre-, or pro- or prepro-protein sequence. The polynucleotide of the invention may also contain at least one non-coding sequence, including for example, but not limited to at least one non-coding 5' and 3' sequence, such as the transcribed but non-translated sequences, termination signals (such as rho-dependent and rho-independent termination signals), ribosome binding sites, Kozak sequences, sequences that stabilize mRNA, introns, and polyadenylation signals. The polynucleotide sequence may also comprise additional coding sequence encoding additional amino acids. For example, a marker sequence that facilitates purification of the fused polypeptide can be encoded. In certain embodiments of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz

et al., *Proc. Natl. Acad. Sci., USA* 86: 821-824 (1989), or an HA peptide tag (Wilson *et al.*, *Cell* 37: 767 (1984), both of which may be useful in purifying polypeptide sequence fused to them. Polynucleotides of the invention also include, but are not limited to, polynucleotides comprising a structural gene and its naturally associated sequences that control gene expression.

The nucleotide sequence encoding BASB063 polypeptide of SEQ ID NO:10 may be identical to the polypeptide encoding sequence contained in nucleotides 1 to 813 of SEQ ID NO:9. Alternatively it may be a sequence, which as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:10.

The term "polynucleotide encoding a polypeptide" as used herein encompasses polynucleotides that include a sequence encoding a polypeptide of the invention, particularly a bacterial polypeptide and more particularly a polypeptide of the *Neisseria meningitidis* BASB063 having an amino acid sequence set out in SEQ ID NO:10. The term also encompasses polynucleotides that include a single continuous region or discontinuous regions encoding the polypeptide (for example, polynucleotides interrupted by integrated phage, an integrated insertion sequence, an integrated vector sequence, an integrated transposon sequence, or due to RNA editing or genomic DNA reorganization) together with additional regions, that also may contain coding and/or non-coding sequences.

The invention further relates to variants of the polynucleotides described herein that encode variants of a polypeptide having a deduced amino acid sequence of SEQ ID NO:10.

Fragments of polynucleotides of the invention may be used, for example, to synthesize full-length polynucleotides of the invention.

Further particularly preferred embodiments are polynucleotides encoding BASB063 variants, that have the amino acid sequence of BASB063 polypeptide of SEQ ID NO:10 in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, modified, deleted and/or added, in any combination. Especially preferred among these are silent

substitutions, additions and deletions that do not alter the properties and activities of BASB063 polypeptide.

Further preferred embodiments of the invention are polynucleotides that are at least 85% identical over their entire length to a polynucleotide encoding BASB063 polypeptide having an amino acid sequence set out in SEQ ID NO:10 and polynucleotides that are complementary to such polynucleotides. In this regard, polynucleotides at least 90% identical over their entire length to the same are particularly preferred, and among these particularly preferred polynucleotides, those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred among those with at least 95%, and among these those with at least 98% and at least 99% are particularly highly preferred, with at least 99% being the more preferred.

Preferred embodiments are polynucleotides encoding polypeptides that retain substantially
15 the same biological function or activity as the mature polypeptide encoded by a DNA of SEQ
ID NO:9.

In accordance with certain preferred embodiments of this invention there are provided polynucleotides that hybridize, particularly under stringent conditions, to BASB063
20 polynucleotide sequences, such as those polynucleotides in SEQ ID NO:9.

The invention further relates to polynucleotides that hybridize to the polynucleotide sequences provided herein. In this regard, the invention especially relates to polynucleotides that hybridize under stringent conditions to the polynucleotides described herein. As herein
25 used, the terms "stringent conditions" and "stringent hybridization conditions" mean hybridization occurring only if there is at least 95% and preferably at least 97% identity between the sequences. A specific example of stringent hybridization conditions is overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution,
30 10% dextran sulfate, and 20 micrograms/ml of denatured, sheared salmon sperm DNA,

5 followed by washing the hybridization support in 0.1x SSC at about 65°C. Hybridization and wash conditions are well known and exemplified in Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), particularly Chapter 11 therein. Solution hybridization may also be used with the polynucleotide sequences provided by the invention.

10 The invention also provides a polynucleotide consisting of or comprising a polynucleotide sequence obtained by screening an appropriate library containing the complete gene for a polynucleotide sequence set forth in SEQ ID NO:9 under stringent hybridization conditions with a probe having the sequence of said polynucleotide sequence set forth in SEQ ID NO:9 or a fragment thereof; and isolating said polynucleotide sequence. Fragments useful for obtaining such a polynucleotide include, for example, probes and primers fully described elsewhere herein.

15 As discussed elsewhere herein regarding polynucleotide assays of the invention, for instance, the polynucleotides of the invention, may be used as a hybridization probe for RNA, cDNA and genomic DNA to isolate full-length cDNAs and genomic clones encoding BASB063 and to isolate cDNA and genomic clones of other genes that have a high identity, particularly high sequence identity, to the BASB063 gene. Such probes generally will comprise at least 15 nucleotide residues or base pairs. Preferably, such probes will have at least 30 nucleotide residues or base pairs and may have at least 50 nucleotide residues or base pairs. Particularly preferred probes will have at least 20 nucleotide residues or base pairs and will have less than 30 nucleotide residues or base pairs.

25 A coding region of a BASB063 gene may be isolated by screening using a DNA sequence provided in SEQ ID NO:9 to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the invention is then used to screen a library of cDNA, genomic DNA or mRNA to determine which members of the library the probe hybridizes to.

It is an object of the invention to provide polynucleotides that encode BASB065 polypeptides, particularly polynucleotides that encode the polypeptide herein designated BASB065.

- 5 In a particularly preferred embodiment of the invention the polynucleotide comprises a region encoding BASB065 polypeptides comprising a sequence set out in SEQ ID NO:11 which includes a full length gene, or a variant thereof.

10 The BASB065 polynucleotide provided in SEQ ID NO:11 is the BASB065 polynucleotide from *Neisseria meningitidis* strains ATCC13090.

As a further aspect of the invention there are provided isolated nucleic acid molecules encoding and/or expressing BASB065 polypeptides and polynucleotides, particularly *Neisseria meningitidis* BASB065 polypeptides and polynucleotides, including, for example, unprocessed RNAs, ribozyme RNAs, mRNAs, cDNAs, genomic DNAs, B- and Z-DNAs. Further embodiments of the invention include biologically, diagnostically, prophylactically, clinically or therapeutically useful polynucleotides and polypeptides, and variants thereof, and compositions comprising the same.

- Another aspect of the invention relates to isolated polynucleotides, including at least one full length gene, that encodes a BASB065 polypeptide having a deduced amino acid sequence of SEQ ID NO:12 and polynucleotides closely related thereto and variants thereof.

25 In another particularly preferred embodiment of the invention there is a BASB065 polypeptide from *Neisseria meningitidis* comprising or consisting of an amino acid sequence of SEQ ID NO:12 or a variant thereof.

Using the information provided herein, such as a polynucleotide sequence set out in SEQ ID NO:11 a polynucleotide of the invention encoding BASB065 polypeptide may be obtained using standard cloning and screening methods, such as those for cloning and sequencing

chromosomal DNA fragments from bacteria using *Neisseria meningitidis* cells as starting material, followed by obtaining a full length clone. For example, to obtain a polynucleotide sequence of the invention, such as a polynucleotide sequence given in SEQ ID NO:11 typically a library of clones of chromosomal DNA of *Neisseria meningitidis* in *E.coli* or
5 some other suitable host is probed with a radiolabeled oligonucleotide, preferably a 17-mer or longer, derived from a partial sequence. Clones carrying DNA identical to that of the probe can then be distinguished using stringent hybridization conditions. By sequencing the individual clones thus identified by hybridization with sequencing primers designed from the original polypeptide or polynucleotide sequence it is then possible to extend the
10 polynucleotide sequence in both directions to determine a full length gene sequence. Conveniently, such sequencing is performed, for example, using denatured double stranded DNA prepared from a plasmid clone. Suitable techniques are described by Maniatis, T., Fritsch, E.F. and Sambrook et al., *MOLECULAR CLONING, A LABORATORY MANUAL*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989). (see
15 in particular Screening By Hybridization 1.90 and Sequencing Denatured Double-Stranded DNA Templates 13.70). Direct genomic DNA sequencing may also be performed to obtain a full length gene sequence. Illustrative of the invention, each polynucleotide set out in SEQ ID NO:11 was discovered in a DNA library derived from *Neisseria meningitidis*.

20 Moreover, the DNA sequence set out in SEQ ID NO:11 contains an open reading frame encoding a protein having about the number of amino acid residues set forth in SEQ ID NO:12 with a deduced molecular weight that can be calculated using amino acid residue molecular weight values well known to those skilled in the art.

25 The polynucleotide of SEQ ID NO:11, between the start codon at nucleotide number 1 and the stop codon which begins at nucleotide number 715 of SEQ ID NO:11, encodes the polypeptide of SEQ ID NO:12.

In a further aspect, the present invention provides for an isolated polynucleotide comprising
30 or consisting of:

- (a) a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:11 over the entire length of SEQ ID NO:11; or
- (b) a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or 100% exact, to the amino acid sequence of SEQ ID NO:12 over the entire length of SEQ ID NO:12.

A polynucleotide encoding a polypeptide of the present invention, including homologs and orthologs from species other than *Neisseria meningitidis*, may be obtained by a process which comprises the steps of screening an appropriate library under stringent hybridization conditions (for example, using a temperature in the range of 45 – 65°C and an SDS concentration from 0.1 – 1%) with a labeled or detectable probe consisting of or comprising the sequence of SEQ ID NO:11 or a fragment thereof; and isolating a full-length gene and/or genomic clones containing said polynucleotide sequence.

The invention provides a polynucleotide sequence identical over its entire length to a coding sequence (open reading frame) in SEQ ID NO:11. Also provided by the invention is a coding sequence for a mature polypeptide or a fragment thereof, by itself as well as a coding sequence for a mature polypeptide or a fragment in reading frame with another coding sequence, such as a sequence encoding a leader or secretory sequence, a pre-, or pro- or prepro-protein sequence. The polynucleotide of the invention may also contain at least one non-coding sequence, including for example, but not limited to at least one non-coding 5' and 3' sequence, such as the transcribed but non-translated sequences, termination signals (such as rho-dependent and rho-independent termination signals), ribosome binding sites, Kozak sequences, sequences that stabilize mRNA, introns, and polyadenylation signals. The polynucleotide sequence may also comprise additional coding sequence encoding additional amino acids. For example, a marker sequence that facilitates purification of the fused polypeptide can be encoded. In certain embodiments of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz

et al., *Proc. Natl. Acad. Sci., USA* 86: 821-824 (1989), or an HA peptide tag (Wilson *et al.*, *Cell* 37: 767 (1984), both of which may be useful in purifying polypeptide sequence fused to them. Polynucleotides of the invention also include, but are not limited to, polynucleotides comprising a structural gene and its naturally associated sequences that control gene expression.

The nucleotide sequence encoding BASB065 polypeptide of SEQ ID NO:12 may be identical to the polypeptide encoding sequence contained in nucleotides 1 to 714 of SEQ ID NO:11. Alternatively it may be a sequence, which as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:12.

The term "polynucleotide encoding a polypeptide" as used herein encompasses polynucleotides that include a sequence encoding a polypeptide of the invention, particularly a bacterial polypeptide and more particularly a polypeptide of the *Neisseria meningitidis* BASB065 having an amino acid sequence set out in SEQ ID NO:12. The term also encompasses polynucleotides that include a single continuous region or discontinuous regions encoding the polypeptide (for example, polynucleotides interrupted by integrated phage, an integrated insertion sequence, an integrated vector sequence, an integrated transposon sequence, or due to RNA editing or genomic DNA reorganization) together with additional regions, that also may contain coding and/or non-coding sequences.

The invention further relates to variants of the polynucleotides described herein that encode variants of a polypeptide having a deduced amino acid sequence of SEQ ID NO:12.

Fragments of polynucleotides of the invention may be used, for example, to synthesize full-length polynucleotides of the invention.

Further particularly preferred embodiments are polynucleotides encoding BASB065 variants, that have the amino acid sequence of BASB065 polypeptide of SEQ ID NO:12 in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, modified, deleted and/or added, in any combination. Especially preferred among these are silent

substitutions, additions and deletions, that do not alter the properties and activities of BASB065 polypeptide.

Further preferred embodiments of the invention are polynucleotides that are at least 85% identical over their entire length to a polynucleotide encoding BASB065 polypeptide having an amino acid sequence set out in SEQ ID NO:12 and polynucleotides that are complementary to such polynucleotides. In this regard, polynucleotides at least 90% identical over their entire length to the same are particularly preferred, and among these particularly preferred polynucleotides, those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred among those with at least 95%, and among these those with at least 98% and at least 99% are particularly highly preferred, with at least 99% being the more preferred.

Preferred embodiments are polynucleotides encoding polypeptides that retain substantially the same biological function or activity as the mature polypeptide encoded by a DNA of SEQ ID NO:11.

In accordance with certain preferred embodiments of this invention there are provided polynucleotides that hybridize, particularly under stringent conditions, to BASB065 polynucleotide sequences, such as those polynucleotides in SEQ ID NO:11.

The invention further relates to polynucleotides that hybridize to the polynucleotide sequences provided herein. In this regard, the invention especially relates to polynucleotides that hybridize under stringent conditions to the polynucleotides described herein. As herein used, the terms "stringent conditions" and "stringent hybridization conditions" mean hybridization occurring only if there is at least 95% and preferably at least 97% identity between the sequences. A specific example of stringent hybridization conditions is overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 micrograms/ml of denatured, sheared salmon sperm DNA,

5 followed by washing the hybridization support in 0.1x SSC at about 65°C. Hybridization and wash conditions are well known and exemplified in Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), particularly Chapter 11 therein. Solution hybridization may also be used with the polynucleotide sequences provided by the invention.

10 The invention also provides a polynucleotide consisting of or comprising a polynucleotide sequence obtained by screening an appropriate library containing the complete gene for a polynucleotide sequence set forth in SEQ ID NO:11 under stringent hybridization conditions with a probe having the sequence of said polynucleotide sequence set forth in SEQ ID NO:11 or a fragment thereof; and isolating said polynucleotide sequence. Fragments useful for obtaining such a polynucleotide include, for example, probes and primers fully described elsewhere herein.

15 As discussed elsewhere herein regarding polynucleotide assays of the invention, for instance, the polynucleotides of the invention, may be used as a hybridization probe for RNA, cDNA and genomic DNA to isolate full-length cDNAs and genomic clones encoding BASB065 and to isolate cDNA and genomic clones of other genes that have a high identity, particularly high sequence identity, to the BASB065 gene. Such probes generally will comprise at least 15
20 nucleotide residues or base pairs. Preferably, such probes will have at least 30 nucleotide residues or base pairs and may have at least 50 nucleotide residues or base pairs. Particularly preferred probes will have at least 20 nucleotide residues or base pairs and will have less than 30 nucleotide residues or base pairs.

25 A coding region of a BASB065 gene may be isolated by screening using a DNA sequence provided in SEQ ID NO:11 to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the invention is then used to screen a library of cDNA, genomic DNA or mRNA to determine which members of the library the probe hybridizes to.

It is an object of the invention to provide polynucleotides that encode BASB066 polypeptides, particularly polynucleotides that encode the polypeptide herein designated BASB066.

- 5 In a particularly preferred embodiment of the invention the polynucleotide comprises a region encoding BASB066 polypeptides comprising a sequence set out in SEQ ID NO:13 which includes a full length gene, or a variant thereof.

- 10 The BASB066 polynucleotide provided in SEQ ID NO:13 is the BASB066 polynucleotide from *Neisseria meningitidis* strains ATCC13090.

- As a further aspect of the invention there are provided isolated nucleic acid molecules encoding and/or expressing BASB066 polypeptides and polynucleotides, particularly *Neisseria meningitidis* BASB066 polypeptides and polynucleotides, including, for
15 example, unprocessed RNAs, ribozyme RNAs, mRNAs, cDNAs, genomic DNAs, B- and Z-DNAs. Further embodiments of the invention include biologically, diagnostically, prophylactically, clinically or therapeutically useful polynucleotides and polypeptides, and variants thereof, and compositions comprising the same.

- 20 Another aspect of the invention relates to isolated polynucleotides, including at least one full length gene, that encodes a BASB066 polypeptide having a deduced amino acid sequence of SEQ ID NO:14 and polynucleotides closely related thereto and variants thereof.

- In another particularly preferred embodiment of the invention there is a BASB066
25 polypeptide from *Neisseria meningitidis* comprising or consisting of an amino acid sequence of SEQ ID NO:14 or a variant thereof.

- Using the information provided herein, such as a polynucleotide sequence set out in SEQ ID NO:13 a polynucleotide of the invention encoding BASB066 polypeptide may be obtained
30 using standard cloning and screening methods, such as those for cloning and sequencing

chromosomal DNA fragments from bacteria using *Neisseria meningitidis* cells as starting material, followed by obtaining a full length clone. For example, to obtain a polynucleotide sequence of the invention, such as a polynucleotide sequence given in SEQ ID NO:13 typically a library of clones of chromosomal DNA of *Neisseria meningitidis* in *E.coli* or
5 some other suitable host is probed with a radiolabeled oligonucleotide, preferably a 17-mer or longer, derived from a partial sequence. Clones carrying DNA identical to that of the probe can then be distinguished using stringent hybridization conditions. By sequencing the individual clones thus identified by hybridization with sequencing primers designed from the original polypeptide or polynucleotide sequence it is then possible to extend the
10 polynucleotide sequence in both directions to determine a full length gene sequence. Conveniently, such sequencing is performed, for example, using denatured double stranded DNA prepared from a plasmid clone. Suitable techniques are described by Maniatis, T., Fritsch, E.F. and Sambrook et al., *MOLECULAR CLONING, A LABORATORY MANUAL*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989). (see
15 in particular Screening By Hybridization 1.90 and Sequencing Denatured Double-Stranded DNA Templates 13.70). Direct genomic DNA sequencing may also be performed to obtain a full length gene sequence. Illustrative of the invention, each polynucleotide set out in SEQ ID NO:13 was discovered in a DNA library derived from *Neisseria meningitidis*.

20 Moreover, the DNA sequence set out in SEQ ID NO:13 contains an open reading frame encoding a protein having about the number of amino acid residues set forth in SEQ ID NO:14 with a deduced molecular weight that can be calculated using amino acid residue molecular weight values well known to those skilled in the art.

25 The polynucleotide of SEQ ID NO:13, between the start codon at nucleotide number 1 and the stop codon which begins at nucleotide number 1174 of SEQ ID NO:13, encodes the polypeptide of SEQ ID NO:14.

In a further aspect, the present invention provides for an isolated polynucleotide comprising
30 or consisting of:

- (a) a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:13 over the entire length of SEQ ID NO:13; or
- (b) a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or 100% exact, to the amino acid sequence of SEQ ID NO:14 over the entire length of SEQ ID NO:14.

A polynucleotide encoding a polypeptide of the present invention, including homologs and orthologs from species other than *Neisseria meningitidis*, may be obtained by a process which comprises the steps of screening an appropriate library under stringent hybridization conditions (for example, using a temperature in the range of 45 – 65°C and an SDS concentration from 0.1 – 1%) with a labeled or detectable probe consisting of or comprising the sequence of SEQ ID NO:13 or a fragment thereof; and isolating a full-length gene and/or genomic clones containing said polynucleotide sequence.

The invention provides a polynucleotide sequence identical over its entire length to a coding sequence (open reading frame) in SEQ ID NO:13. Also provided by the invention is a coding sequence for a mature polypeptide or a fragment thereof, by itself as well as a coding sequence for a mature polypeptide or a fragment in reading frame with another coding sequence, such as a sequence encoding a leader or secretory sequence, a pre-, or pro- or prepro-protein sequence. The polynucleotide of the invention may also contain at least one non-coding sequence, including for example, but not limited to at least one non-coding 5' and 3' sequence, such as the transcribed but non-translated sequences, termination signals (such as rho-dependent and rho-independent termination signals), ribosome binding sites, Kozak sequences, sequences that stabilize mRNA, introns, and polyadenylation signals. The polynucleotide sequence may also comprise additional coding sequence encoding additional amino acids. For example, a marker sequence that facilitates purification of the fused polypeptide can be encoded. In certain embodiments of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz

5 *et al.*, *Proc. Natl. Acad. Sci., USA* 86: 821-824 (1989), or an HA peptide tag (Wilson *et al.*, *Cell* 37: 767 (1984), both of which may be useful in purifying polypeptide sequence fused to them. Polynucleotides of the invention also include, but are not limited to, polynucleotides comprising a structural gene and its naturally associated sequences that control gene expression.

10 The nucleotide sequence encoding BASB066 polypeptide of SEQ ID NO:14 may be identical to the polypeptide encoding sequence contained in nucleotides 1 to 1173 of SEQ ID NO:13. Alternatively it may be a sequence, which as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:14. The term "polynucleotide encoding a polypeptide" as used herein encompasses polynucleotides that include a sequence encoding a polypeptide of the invention, particularly a bacterial polypeptide and more particularly a polypeptide of the *Neisseria meningitidis* BASB066 having an amino acid sequence set out in SEQ ID NO:14. The term also

15 encompasses polynucleotides that include a single continuous region or discontinuous regions encoding the polypeptide (for example, polynucleotides interrupted by integrated phage, an integrated insertion sequence, an integrated vector sequence, an integrated transposon sequence, or due to RNA editing or genomic DNA reorganization) together with additional regions, that also may contain coding and/or non-coding sequences.

20

The invention further relates to variants of the polynucleotides described herein that encode variants of a polypeptide having a deduced amino acid sequence of SEQ ID NO:14.

Fragments of polynucleotides of the invention may be used, for example, to synthesize full-length polynucleotides of the invention.

25

Further particularly preferred embodiments are polynucleotides encoding BASB066 variants, that have the amino acid sequence of BASB066 polypeptide of SEQ ID NO:14 in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, modified, deleted and/or added, in any combination. Especially preferred among these are silent

substitutions, additions and deletions, that do not alter the properties and activities of BASB066 polypeptide.

Further preferred embodiments of the invention are polynucleotides that are at least 85% identical over their entire length to a polynucleotide encoding BASB066 polypeptide having an amino acid sequence set out in SEQ ID NO:14 and polynucleotides that are complementary to such polynucleotides. In this regard, polynucleotides at least 90% identical over their entire length to the same are particularly preferred, and among these particularly preferred polynucleotides, those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred among those with at least 95%, and among these those with at least 98% and at least 99% are particularly highly preferred, with at least 99% being the more preferred.

Preferred embodiments are polynucleotides encoding polypeptides that retain substantially
15 the same biological function or activity as the mature polypeptide encoded by a DNA of SEQ
ID NO:13.

In accordance with certain preferred embodiments of this invention there are provided polynucleotides that hybridize, particularly under stringent conditions, to BASB066
20 polynucleotide sequences, such as those polynucleotides in SEQ ID NO:13.

The invention further relates to polynucleotides that hybridize to the polynucleotide sequences provided herein. In this regard, the invention especially relates to polynucleotides that hybridize under stringent conditions to the polynucleotides described herein. As herein
25 used, the terms "stringent conditions" and "stringent hybridization conditions" mean hybridization occurring only if there is at least 95% and preferably at least 97% identity between the sequences. A specific example of stringent hybridization conditions is overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution,
30 10% dextran sulfate, and 20 micrograms/ml of denatured, sheared salmon sperm DNA,

followed by washing the hybridization support in 0.1x SSC at about 65°C. Hybridization and wash conditions are well known and exemplified in Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), particularly Chapter 11 therein. Solution hybridization may also be used with the polynucleotide sequences provided by the invention.

The invention also provides a polynucleotide consisting of or comprising a polynucleotide sequence obtained by screening an appropriate library containing the complete gene for a polynucleotide sequence set forth in SEQ ID NO:13 under stringent hybridization conditions with a probe having the sequence of said polynucleotide sequence set forth in SEQ ID NO:13 or a fragment thereof; and isolating said polynucleotide sequence. Fragments useful for obtaining such a polynucleotide include, for example, probes and primers fully described elsewhere herein.

As discussed elsewhere herein regarding polynucleotide assays of the invention, for instance, the polynucleotides of the invention, may be used as a hybridization probe for RNA, cDNA and genomic DNA to isolate full-length cDNAs and genomic clones encoding BASB066 and to isolate cDNA and genomic clones of other genes that have a high identity, particularly high sequence identity, to the BASB066 gene. Such probes generally will comprise at least 15 nucleotide residues or base pairs. Preferably, such probes will have at least 30 nucleotide residues or base pairs and may have at least 50 nucleotide residues or base pairs. Particularly preferred probes will have at least 20 nucleotide residues or base pairs and will have less than 30 nucleotide residues or base pairs.

25 A coding region of a BASB066 gene may be isolated by screening using a DNA sequence provided in SEQ ID NO:13 to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the invention is then used to screen a library of cDNA, genomic DNA or mRNA to determine which members of the library the probe hybridizes to.

It is an object of the invention to provide polynucleotides that encode BASB071 polypeptides, particularly polynucleotides that encode the polypeptide herein designated BASB071.

- 5 In a particularly preferred embodiment of the invention the polynucleotide comprises a region encoding BASB071 polypeptides comprising a sequence set out in SEQ ID NO:15 which includes a full length gene, or a variant thereof.

- 10 The BASB071 polynucleotide provided in SEQ ID NO:15 is the BASB071 polynucleotide from *Neisseria meningitidis* strains ATCC13090.

- As a further aspect of the invention there are provided isolated nucleic acid molecules encoding and/or expressing BASB071 polypeptides and polynucleotides, particularly *Neisseria meningitidis* BASB071 polypeptides and polynucleotides, including, for
15 example, unprocessed RNAs, ribozyme RNAs, mRNAs, cDNAs, genomic DNAs, B- and Z-DNAs. Further embodiments of the invention include biologically, diagnostically, prophylactically, clinically or therapeutically useful polynucleotides and polypeptides, and variants thereof, and compositions comprising the same.

- 20 Another aspect of the invention relates to isolated polynucleotides, including at least one full length gene, that encodes a BASB071 polypeptide having a deduced amino acid sequence of SEQ ID NO:16 and polynucleotides closely related thereto and variants thereof.

- In another particularly preferred embodiment of the invention there is a BASB071
25 polypeptide from *Neisseria meningitidis* comprising or consisting of an amino acid sequence of SEQ ID NO:16 or a variant thereof.

- Using the information provided herein, such as a polynucleotide sequence set out in SEQ ID NO:15 a polynucleotide of the invention encoding BASB071 polypeptide may be obtained
30 using standard cloning and screening methods, such as those for cloning and sequencing

chromosomal DNA fragments from bacteria using *Neisseria meningitidis* cells as starting material, followed by obtaining a full length clone. For example, to obtain a polynucleotide sequence of the invention, such as a polynucleotide sequence given in SEQ ID NO:15 typically a library of clones of chromosomal DNA of *Neisseria meningitidis* in *E.coli* or some other suitable host is probed with a radiolabeled oligonucleotide, preferably a 17-mer or longer, derived from a partial sequence. Clones carrying DNA identical to that of the probe can then be distinguished using stringent hybridization conditions. By sequencing the individual clones thus identified by hybridization with sequencing primers designed from the original polypeptide or polynucleotide sequence it is then possible to extend the polynucleotide sequence in both directions to determine a full length gene sequence. Conveniently, such sequencing is performed, for example, using denatured double stranded DNA prepared from a plasmid clone. Suitable techniques are described by Maniatis, T., Fritsch, E.F. and Sambrook et al., *MOLECULAR CLONING, A LABORATORY MANUAL*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989). (see in particular Screening By Hybridization 1.90 and Sequencing Denatured Double-Stranded DNA Templates 13.70). Direct genomic DNA sequencing may also be performed to obtain a full length gene sequence. Illustrative of the invention, each polynucleotide set out in SEQ ID NO:15 was discovered in a DNA library derived from *Neisseria meningitidis*.

Moreover, the DNA sequence set out in SEQ ID NO:15 contains an open reading frame encoding a protein having about the number of amino acid residues set forth in SEQ ID NO:16 with a deduced molecular weight that can be calculated using amino acid residue molecular weight values well known to those skilled in the art.

The polynucleotide of SEQ ID NO:15, between the start codon at nucleotide number 1 and the stop codon which begins at nucleotide number 805 of SEQ ID NO:15, encodes the polypeptide of SEQ ID NO:16.

In a further aspect, the present invention provides for an isolated polynucleotide comprising or consisting of:

- (a) a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:15 over the entire length of SEQ ID NO:15; or
- (b) a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or 100% exact, to the amino acid sequence of SEQ ID NO:16 over the entire length of SEQ ID NO:16.

A polynucleotide encoding a polypeptide of the present invention, including homologs and orthologs from species other than *Neisseria meningitidis*, may be obtained by a process which comprises the steps of screening an appropriate library under stringent hybridization conditions (for example, using a temperature in the range of 45 – 65°C and an SDS concentration from 0.1 – 1%) with a labeled or detectable probe consisting of or comprising the sequence of SEQ ID NO:15 or a fragment thereof; and isolating a full-length gene and/or genomic clones containing said polynucleotide sequence.

The invention provides a polynucleotide sequence identical over its entire length to a coding sequence (open reading frame) in SEQ ID NO:15. Also provided by the invention is a coding sequence for a mature polypeptide or a fragment thereof, by itself as well as a coding sequence for a mature polypeptide or a fragment in reading frame with another coding sequence, such as a sequence encoding a leader or secretory sequence, a pre-, or pro- or prepro-protein sequence. The polynucleotide of the invention may also contain at least one non-coding sequence, including for example, but not limited to at least one non-coding 5' and 3' sequence, such as the transcribed but non-translated sequences, termination signals (such as rho-dependent and rho-independent termination signals), ribosome binding sites, Kozak sequences, sequences that stabilize mRNA, introns, and polyadenylation signals. The polynucleotide sequence may also comprise additional coding sequence encoding additional amino acids. For example, a marker sequence that facilitates purification of the fused polypeptide can be encoded. In certain embodiments of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz

substitutions, additions and deletions, that do not alter the properties and activities of BASB071 polypeptide.

Further preferred embodiments of the invention are polynucleotides that are at least 85% identical over their entire length to a polynucleotide encoding BASB071 polypeptide having an amino acid sequence set out in SEQ ID NO:16 and polynucleotides that are complementary to such polynucleotides. In this regard, polynucleotides at least 90% identical over their entire length to the same are particularly preferred, and among these particularly preferred polynucleotides, those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred among those with at least 95%, and among these those with at least 98% and at least 99% are particularly highly preferred, with at least 99% being the more preferred.

Preferred embodiments are polynucleotides encoding polypeptides that retain substantially the same biological function or activity as the mature polypeptide encoded by a DNA of SEQ ID NO:15.

In accordance with certain preferred embodiments of this invention there are provided polynucleotides that hybridize, particularly under stringent conditions, to BASB071 polynucleotide sequences, such as those polynucleotides in SEQ ID NO:15.

The invention further relates to polynucleotides that hybridize to the polynucleotide sequences provided herein. In this regard, the invention especially relates to polynucleotides that hybridize under stringent conditions to the polynucleotides described herein. As herein used, the terms "stringent conditions" and "stringent hybridization conditions" mean hybridization occurring only if there is at least 95% and preferably at least 97% identity between the sequences. A specific example of stringent hybridization conditions is overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 micrograms/ml of denatured, sheared salmon sperm DNA,

The invention also provides polynucleotides that encode a polypeptide that is the mature protein plus additional amino or carboxyl-terminal amino acids, or amino acids interior to the mature polypeptide (when the mature form has more than one polypeptide chain, for instance). Such sequences may play a role in processing of a protein from precursor to a mature form, may allow protein transport, may lengthen or shorten protein half-life or may facilitate manipulation of a protein for assay or production, among other things. As generally is the case *in vivo*, the additional amino acids may be processed away from the mature protein by cellular enzymes.

For each and every polynucleotide of the invention there is provided a polynucleotide complementary to it. It is preferred that these complementary polynucleotides are fully complementary to each polynucleotide with which they are complementary.

A precursor protein, having a mature form of the polypeptide fused to one or more prosequences may be an inactive form of the polypeptide. When prosequences are removed such inactive precursors generally are activated. Some or all of the prosequences may be removed before activation. Generally, such precursors are called proproteins.

In addition to the standard A, G, C, T/U representations for nucleotides, the term "N" may also be used in describing certain polynucleotides of the invention. "N" means that any of the four DNA or RNA nucleotides may appear at such a designated position in the DNA or RNA sequence, except it is preferred that N is not a nucleic acid that when taken in combination with adjacent nucleotide positions, when read in the correct reading frame, would have the effect of generating a premature termination codon in such reading frame.

In sum, a polynucleotide of the invention may encode a mature protein, a mature protein plus a leader sequence (which may be referred to as a preprotein), a precursor of a mature protein having one or more prosequences that are not the leader sequences of a preprotein, or a preproprotein, which is a precursor to a proprotein, having a leader sequence and one or more

prosequences, which generally are removed during processing steps that produce active and mature forms of the polypeptide.

In accordance with an aspect of the invention, there is provided the use of a polynucleotide
5 of the invention for therapeutic or prophylactic purposes, in particular genetic immunization.

The use of a polynucleotide of the invention in genetic immunization will preferably employ a suitable delivery method such as direct injection of plasmid DNA into muscles
10 (Wolff *et al.*, *Hum Mol Genet* (1992) 1: 363, Manthorpe *et al.*, *Hum. Gene Ther.* (1983) 4: 419), delivery of DNA complexed with specific protein carriers (Wu *et al.*, *J Biol Chem.* (1989) 264: 16985), coprecipitation of DNA with calcium phosphate (Benvenisty & Reshef, *PNAS USA*, (1986) 83: 9551), encapsulation of DNA in various forms of liposomes (Kaneda *et al.*, *Science* (1989) 243: 375), particle bombardment (Tang *et al.*, *Nature* (1992)
15 356:152, Eisenbraun *et al.*, *DNA Cell Biol* (1993) 12: 791) and *in vivo* infection using cloned retroviral vectors (Seeger *et al.*, *PNAS USA* (1984) 81: 5849).

Vectors, Host Cells, Expression Systems

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The invention also relates to vectors that comprise a polynucleotide or polynucleotides of the invention, host cells that are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA
25 constructs of the invention.

Recombinant polypeptides of the present invention may be prepared by processes well known in those skilled in the art from genetically engineered host cells comprising expression systems. Accordingly, in a further aspect, the present invention relates to expression systems
30 that comprise a polynucleotide or polynucleotides of the present invention, to host cells which

For recombinant production of the polypeptides of the invention, host cells can be genetically engineered to incorporate expression systems or portions thereof or polynucleotides of the invention. Introduction of a polynucleotide into the host cell can be effected by methods described in many standard laboratory manuals, such as Davis, *et al.*, *BASIC METHODS IN MOLECULAR BIOLOGY*, (1986) and Sambrook, *et al.*, *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989), such as, calcium phosphate transfection, DEAE-dextran mediated transfection, transvection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction and infection.

Representative examples of appropriate hosts include bacterial cells, such as cells of streptococci, staphylococci, enterococci, *E. coli*, streptomyces, cyanobacteria, *Bacillus subtilis*, *Moraxella catarrhalis*, *Haemophilus influenzae* and *Neisseria meningitidis*; fungal cells, such as cells of a yeast, *Kluveromyces*, *Saccharomyces*, a basidiomycete, *Candida albicans* and *Aspergillus*; insect cells such as cells of *Drosophila* S2 and *Spodoptera* Sf9; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, 293, CV-1 and Bowes melanoma cells; and plant cells, such as cells of a gymnosperm or angiosperm.

A great variety of expression systems can be used to produce the polypeptides of the invention. Such vectors include, among others, chromosomal-, episomal- and virus-derived vectors, for example, vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses, picornaviruses, retroviruses, and alphaviruses and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression system constructs may contain control regions that regulate as well as engender

expression. Generally, any system or vector suitable to maintain, propagate or express polynucleotides and/or to express a polypeptide in a host may be used for expression in this regard. The appropriate DNA sequence may be inserted into the expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook *et al.*, *MOLECULAR CLONING, A LABORATORY MANUAL*, (*supra*).

In recombinant expression systems in eukaryotes, for secretion of a translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the expressed polypeptide. These signals may be endogenous to the polypeptide or they may be heterologous signals.

Polypeptides of the present invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, ion metal affinity chromatography (IMAC) is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during intracellular synthesis, isolation and or purification.

The expression system may also be a recombinant live microorganism, such as a virus or bacterium. The gene of interest can be inserted into the genome of a live recombinant virus or bacterium. Inoculation and *in vivo* infection with this live vector will lead to *in vivo* expression of the antigen and induction of immune responses. Viruses and bacteria used for this purpose are for instance: poxviruses (e.g; vaccinia, fowlpox, canarypox), alphaviruses (Sindbis virus, Semliki Forest Virus, Venezuelan Equine Encephalitis Virus), adenoviruses, adeno-associated virus, picornaviruses (poliovirus, rhinovirus), herpesviruses (varicella zoster virus, etc), *Listeria*, *Salmonella*, *Shigella*, *Neisseria*, BCG.

These viruses and bacteria can be virulent, or attenuated in various ways in order to obtain live vaccines. Such live vaccines also form part of the invention.

Diagnostic, Prognostic, Serotyping and Mutation Assays

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This invention is also related to the use of BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polynucleotides and polypeptides of the invention for use as diagnostic reagents. Detection of BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polynucleotides and/or
10 polypeptides in a eukaryote, particularly a mammal, and especially a human, will provide a diagnostic method for diagnosis of disease, staging of disease or response of an infectious organism to drugs. Eukaryotes, particularly mammals, and especially humans, particularly those infected or suspected to be infected with an organism comprising the BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 gene or
15 protein, may be detected at the nucleic acid or amino acid level by a variety of well known techniques as well as by methods provided herein.

Polypeptides and polynucleotides for prognosis, diagnosis or other analysis may be obtained from a putatively infected and/or infected individual's bodily materials. Polynucleotides from
20 any of these sources, particularly DNA or RNA, may be used directly for detection or may be amplified enzymatically by using PCR or any other amplification technique prior to analysis. RNA, particularly mRNA, cDNA and genomic DNA may also be used in the same ways. Using amplification, characterization of the species and strain of infectious or resident organism present in an individual, may be made by an analysis of the genotype of a selected
25 polynucleotide of the organism. Deletions and insertions can be detected by a change in size of the amplified product in comparison to a genotype of a reference sequence selected from a related organism, preferably a different species of the same genus or a different strain of the same species. Point mutations can be identified by hybridizing amplified DNA to labeled BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071
30 polynucleotide sequences. Perfectly or significantly matched sequences can be distinguished

It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component. Such a kit will be of use in diagnosing a disease or susceptibility to a disease, among others.

- 5 This invention also relates to the use of polynucleotides of the present invention as diagnostic reagents. Detection of a mutated form of a polynucleotide of the invention, preferable, SEQ ID NO:1,3,5,7,9,11,13,15 which is associated with a disease or pathogenicity will provide a diagnostic tool that can add to, or define, a diagnosis of a disease, a prognosis of a course of disease, a determination of a stage of disease, or a susceptibility to a disease, which results
- 10 from under-expression, over-expression or altered expression of the polynucleotide. Organisms, particularly infectious organisms, carrying mutations in such polynucleotide may be detected at the polynucleotide level by a variety of techniques, such as those described elsewhere herein.
- 15 Cells from an organism carrying mutations or polymorphisms (allelic variations) in a polynucleotide and/or polypeptide of the invention may also be detected at the polynucleotide or polypeptide level by a variety of techniques, to allow for serotyping, for example. For example, RT-PCR can be used to detect mutations in the RNA. It is particularly preferred to use RT-PCR in conjunction with automated detection systems, such as, for example,
- 20 GeneScan. RNA, cDNA or genomic DNA may also be used for the same purpose, PCR. As an example, PCR primers complementary to a polynucleotide encoding BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polypeptide can be used to identify and analyze mutations.
- 25 The invention further provides primers with 1, 2, 3 or 4 nucleotides removed from the 5' and/or the 3' end. These primers may be used for, among other things, amplifying BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 DNA and/or RNA isolated from a sample derived from an individual, such as a bodily material. The primers may be used to amplify a polynucleotide isolated from an infected individual,
- 30 such that the polynucleotide may then be subject to various techniques for elucidation of the

presence of a particular polynucleotide sequence or related sequence in an individual. Such a presence may indicate the presence of a pathogen, particularly *Neisseria meningitidis*, and may be useful in diagnosing and/or prognosing disease or a course of disease. A grid comprising a number of variants of the polynucleotide sequence of SEQ ID NO:1,3,5,7,9,11,13,15 are preferred. Also preferred is a grid comprising a number of variants of a polynucleotide sequence encoding the polypeptide sequence of SEQ ID NO:2,4,6,8,10,12,14,16.

Antibodies

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The polypeptides and polynucleotides of the invention or variants thereof, or cells expressing the same can be used as immunogens to produce antibodies immunospecific for such polypeptides or polynucleotides respectively.

15 In certain preferred embodiments of the invention there are provided antibodies against BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polypeptides or polynucleotides.

Antibodies generated against the polypeptides or polynucleotides of the invention can be obtained by administering the polypeptides and/or polynucleotides of the invention, or epitope-bearing fragments of either or both, analogues of either or both, or cells expressing either or both, to an animal, preferably a nonhuman, using routine protocols. For preparation of monoclonal antibodies, any technique known in the art that provides antibodies produced by continuous cell line cultures can be used. Examples include various techniques, such as those in Kohler, G. and Milstein, C., *Nature* 256: 495-497 (1975); Kozbor *et al.*, *Immunology Today* 4: 72 (1983); Cole *et al.*, pg. 77-96 in *MONOCLONAL ANTIBODIES AND CANCER THERAPY*, Alan R. Liss, Inc. (1985).

Techniques for the production of single chain antibodies (U.S. Patent No. 4,946,778) can be adapted to produce single chain antibodies to polypeptides or polynucleotides of this

invention. Also, transgenic mice, or other organisms or animals, such as other mammals, may be used to express humanized antibodies immunospecific to the polypeptides or polynucleotides of the invention.

- 5 Alternatively, phage display technology may be utilized to select antibody genes with binding activities towards a polypeptide of the invention either from repertoires of PCR amplified v-genes of lymphocytes from humans screened for possessing anti- BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 or from naive libraries (McCafferty, *et al.*, (1990), *Nature* 348, 552-554; Marks, *et al.*, (1992) 10 *Biotechnology* 10, 779-783). The affinity of these antibodies can also be improved by, for example, chain shuffling (Clackson *et al.*, (1991) *Nature* 352: 628).

- The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptides or polynucleotides of the invention to purify the polypeptides or 15 polynucleotides by, for example, affinity chromatography.

- Thus, among others, antibodies against BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 -polypeptide or BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 -polynucleotide may 20 be employed to treat infections, particularly bacterial infections.

Polypeptide variants include antigenically, epitopically or immunologically equivalent variants form a particular aspect of this invention.

- 25 Preferably, the antibody or variant thereof is modified to make it less immunogenic in the individual. For example, if the individual is human the antibody may most preferably be "humanized," where the complementarity determining region or regions of the hybridoma-derived antibody has been transplanted into a human monoclonal antibody, for example as described in Jones *et al.* (1986), *Nature* 321, 522-525 or Tempest *et al.*, (1991) 30 *Biotechnology* 9, 266-273.

BASB063, BASB065, BASB066 or BASB071 polypeptide, as hereinbefore described, can also be used for high-throughput screening assays to identify antagonists of the polypeptide of the present invention, as well as of phylogenetically and and/or functionally related polypeptides (see D. Bennett *et al.*, J Mol Recognition, 8:52-58 (1995); and K. Johanson *et al.*, J Biol Chem, 270(16):9459-9471 (1995)).

The polynucleotides, polypeptides and antibodies that bind to and/or interact with a polypeptide of the present invention may also be used to configure screening methods for detecting the effect of added compounds on the production of mRNA and/or polypeptide in cells. For example, an ELISA assay may be constructed for measuring secreted or cell associated levels of polypeptide using monoclonal and polyclonal antibodies by standard methods known in the art. This can be used to discover agents which may inhibit or enhance the production of polypeptide (also called antagonist or agonist, respectively) from suitably manipulated cells or tissues.

The invention also provides a method of screening compounds to identify those which enhance (agonist) or block (antagonist) the action of BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polypeptides or polynucleotides, particularly those compounds that are bacteristatic and/or bactericidal. The method of screening may involve high-throughput techniques. For example, to screen for agonists or antagonists, a synthetic reaction mix, a cellular compartment, such as a membrane, cell envelope or cell wall, or a preparation of any thereof, comprising BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polypeptide and a labeled substrate or ligand of such polypeptide is incubated in the absence or the presence of a candidate molecule that may be a BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 agonist or antagonist. The ability of the candidate molecule to agonize or antagonize the BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polypeptide is reflected in decreased binding of the labeled ligand or decreased production of product from such substrate. Molecules that bind gratuitously, *i.e.*, without inducing the effects of BASB051, BASB057, BASB060,

BASB061, BASB063, BASB065, BASB066 or BASB071 polypeptide are most likely to be good antagonists. Molecules that bind well and, as the case may be, increase the rate of product production from substrate, increase signal transduction, or increase chemical channel activity are agonists. Detection of the rate or level of, as the case may be, production of product from substrate, signal transduction, or chemical channel activity may be enhanced by using a reporter system. Reporter systems that may be useful in this regard include but are not limited to colorimetric, labeled substrate converted into product, a reporter gene that is responsive to changes in BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polynucleotide or polypeptide activity, and binding assays known in the art.

Another example of an assay for BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 agonists is a competitive assay that combines BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 and a potential agonist with BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 -binding molecules, recombinant BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 binding molecules, natural substrates or ligands, or substrate or ligand mimetics, under appropriate conditions for a competitive inhibition assay. BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 can be labeled, such as by radioactivity or a colorimetric compound, such that the number of BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 molecules bound to a binding molecule or converted to product can be determined accurately to assess the effectiveness of the potential antagonist.

Potential antagonists include, among others, small organic molecules, peptides, polypeptides and antibodies that bind to a polynucleotide and/or polypeptide of the invention and thereby inhibit or extinguish its activity or expression. Potential antagonists also may be small organic molecules, a peptide, a polypeptide such as a closely related protein or antibody that binds the same sites on a binding molecule, such as a binding molecule, without inducing BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071

-induced activities, thereby preventing the action or expression of BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polypeptides and/or polynucleotides by excluding BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polypeptides and/or polynucleotides from binding.

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Potential antagonists include a small molecule that binds to and occupies the binding site of the polypeptide thereby preventing binding to cellular binding molecules, such that normal biological activity is prevented. Examples of small molecules include but are not limited to small organic molecules, peptides or peptide-like molecules. Other potential antagonists include antisense molecules (see Okano, *J. Neurochem.* 56: 560 (1991); *OLIGODEOXYNUCLEOTIDES AS ANTISENSE INHIBITORS OF GENE EXPRESSION*, CRC Press, Boca Raton, FL (1988), for a description of these molecules). Preferred potential antagonists include compounds related to and variants of BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071.

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In a further aspect, the present invention relates to genetically engineered soluble fusion proteins comprising a polypeptide of the present invention, or a fragment thereof, and various portions of the constant regions of heavy or light chains of immunoglobulins of various subclasses (IgG, IgM, IgA, IgE). Preferred as an immunoglobulin is the constant part of the heavy chain of human IgG, particularly IgG1, where fusion takes place at the hinge region. In a particular embodiment, the Fc part can be removed simply by incorporation of a cleavage sequence which can be cleaved with blood clotting factor Xa. Furthermore, this invention relates to processes for the preparation of these fusion proteins by genetic engineering, and to the use thereof for drug screening, diagnosis and therapy. A further aspect of the invention also relates to polynucleotides encoding such fusion proteins. Examples of fusion protein technology can be found in International Patent Application Nos. WO94/29458 and WO94/22914.

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Each of the polynucleotide sequences provided herein may be used in the discovery and development of antibacterial compounds. The encoded protein, upon expression, can be

Peptide mimotopes may be designed for a particular purpose by addition, deletion or substitution of elected amino acids. Thus, the peptides may be modified for the purposes of ease of conjugation to a protein carrier. For example, it may be desirable for some chemical conjugation methods to include a terminal cysteine. In addition it may be desirable for peptides conjugated to a protein carrier to include a hydrophobic terminus distal from the conjugated terminus of the peptide, such that the free unconjugated end of the peptide remains associated with the surface of the carrier protein. Thereby presenting the peptide in a conformation which most closely resembles that of the peptide as found in the context of the whole native molecule. For example, the peptides may be altered to have an N-terminal cysteine and a C-terminal hydrophobic amidated tail. Alternatively, the addition or substitution of a D-stereoisomer form of one or more of the amino acids may be performed to create a beneficial derivative, for example to enhance stability of the peptide.

Alternatively, peptide mimotopes may be identified using antibodies which are capable themselves of binding to the polypeptides of the present invention using techniques such as phage display technology (EP 0 552 267 B1). This technique, generates a large number of peptide sequences which mimic the structure of the native peptides and are, therefore, capable of binding to anti-native peptide antibodies, but may not necessarily themselves share significant sequence homology to the native polypeptide.

Vaccines

Another aspect of the invention relates to a method for inducing an immunological response in an individual, particularly a mammal, preferably humans, which comprises inoculating the individual with BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polynucleotide and/or polypeptide, or a fragment or variant thereof, adequate to produce antibody and/ or T cell immune response to protect said individual from infection, particularly bacterial infection and most particularly

Neisseria meningitidis infection. Also provided are methods whereby such immunological response slows bacterial replication.

Yet another aspect of the invention relates to a method of inducing immunological response
 5 in an individual which comprises delivering to such individual a nucleic acid vector, sequence or ribozyme to direct expression of BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polynucleotide and/or polypeptide, or a fragment or a variant thereof, for expressing BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polynucleotide and/or polypeptide, or a
 10 fragment or a variant thereof *in vivo* in order to induce an immunological response, such as, to produce antibody and/ or T cell immune response, including, for example, cytokine-producing T cells or cytotoxic T cells, to protect said individual, preferably a human, from disease, whether that disease is already established within the individual or not. One example of administering the gene is by accelerating it into the desired cells as a coating on
 15 particles or otherwise. Such nucleic acid vector may comprise DNA, RNA, a ribozyme, a modified nucleic acid, a DNA/RNA hybrid, a DNA-protein complex or an RNA-protein complex.

A further aspect of the invention relates to an immunological composition that when
 20 introduced into an individual, preferably a human, capable of having induced within it an immunological response, induces an immunological response in such individual to a BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polynucleotide and/or polypeptide encoded therefrom, wherein the composition comprises a recombinant BASB051, BASB057, BASB060, BASB061, BASB063, BASB065,
 25 BASB066 or BASB071 polynucleotide and/or polypeptide encoded therefrom and/or comprises DNA and/or RNA which encodes and expresses an antigen of said BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polynucleotide, polypeptide encoded therefrom, or other polypeptide of the invention. The immunological response may be used therapeutically or prophylactically and may take the

form of antibody immunity and/or cellular immunity, such as cellular immunity arising from CTL or CD4+ T cells.

A BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polypeptide or a fragment thereof may be fused with co-protein or chemical moiety which may or may not by itself produce antibodies, but which is capable of stabilizing the first protein and producing a fused or modified protein which will have antigenic and/or immunogenic properties, and preferably protective properties. Thus fused recombinant protein, preferably further comprises an antigenic co-protein, such as lipoprotein D from *Haemophilus influenzae*, Glutathione-S-transferase (GST) or beta-galactosidase, or any other relatively large co-protein which solubilizes the protein and facilitates production and purification thereof. Moreover, the co-protein may act as an adjuvant in the sense of providing a generalized stimulation of the immune system of the organism receiving the protein. The co-protein may be attached to either the amino- or carboxy-terminus of the first protein.

In a vaccine composition according to the invention, a BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polypeptide and/or polynucleotide, or a fragment, or a mimotope, or a variant thereof may be present in a vector, such as the live recombinant vectors described above for example live bacterial vectors.

Also suitable are non-live vectors for the BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polypeptide, for example bacterial outer-membrane vesicles or “blebs”. OM blebs are derived from the outer membrane of the two-layer membrane of Gram-negative bacteria and have been documented in many Gram-negative bacteria (Zhou, L *et al.* 1998. *FEMS Microbiol. Lett.* 163:223-228) including *C. trachomatis* and *C. psittaci*. A non-exhaustive list of bacterial pathogens reported to produce blebs also includes: *Bordetella pertussis*, *Borrelia burgdorferi*, *Brucella melitensis*, *Brucella ovis*, *Escherichia coli*, *Haemophilus influenza*, *Legionella pneumophila*.

Neisseria gonorrhoeae, *Neisseria meningitidis*, *Pseudomonas aeruginosa* and *Yersinia enterocolitica*.

Blebs have the advantage of providing outer-membrane proteins in their native
 5 conformation and are thus particularly useful for vaccines. Blebs can also be improved for
 vaccine use by engineering the bacterium so as to modify the expression of one or more
 molecules at the outer membrane. Thus for example the expression of a desired
 immunogenic protein at the outer membrane, such as the BASB051, BASB057, BASB060,
 BASB061, BASB063, BASB065, BASB066 or BASB071 polypeptide, can be introduced or
 10 upregulated (e.g. by altering the promoter). Instead or in addition, the expression of outer-
 membrane molecules which are either not relevant (e.g. unprotective antigens or
 immunodominant but variable proteins) or detrimental (e.g. toxic molecules such as LPS,
 or potential inducers of an autoimmune response) can be downregulated. These approaches
 are discussed in more detail below.

15 The non-coding flanking regions of the BASB051, BASB057, BASB060, BASB061,
 BASB063, BASB065, BASB066 or BASB071 gene contains regulatory elements important
 in the expression of the gene. This regulation takes place both at the transcriptional and
 translational level. The sequence of these regions, either upstream or downstream of the
 20 open reading frame of the gene, can be obtained by DNA sequencing. This sequence
 information allows the determination of potential regulatory motifs such as the different
 promoter elements, terminator sequences, inducible sequence elements, repressors,
 elements responsible for phase variation, the shine-dalgarno sequence, regions with
 potential secondary structure involved in regulation, as well as other types of regulatory
 25 motifs or sequences.

This sequence information allows the modulation of the natural expression of the
 BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071
 gene. The upregulation of the gene expression may be accomplished by altering the
 30 promoter, the shine-dalgarno sequence, potential repressor or operator elements, or any

other elements involved. Likewise, downregulation of expression can be achieved by similar types of modification. Alternatively, by changing phase variation sequences, the expression of the gene can be put under phase variation control, or it may be uncoupled from this regulation. In another approach, the expression of the gene can be put under the control of one or more inducible elements allowing regulated expression. Examples of such regulation include, but are not limited to, induction by temperature shift, addition of inductor substrates like selected carbohydrates or their derivatives, trace elements, vitamins, co-factors, metal ions, etc.

- Such modifications as described above can be introduced by several different means. The modification of sequences involved in gene expression can be carried out *in vivo* by random mutagenesis followed by selection for the desired phenotype. Another approach consists in isolating the region of interest and modifying it by random mutagenesis, or site-directed replacement, insertion or deletion mutagenesis. The modified region can then be reintroduced into the bacterial genome by homologous recombination, and the effect on gene expression can be assessed. In another approach, the sequence knowledge of the region of interest can be used to replace or delete all or part of the natural regulatory sequences. In this case, the regulatory region targeted is isolated and modified so as to contain the regulatory elements from another gene, a combination of regulatory elements from different genes, a synthetic regulatory region, or any other regulatory region, or to delete selected parts of the wild-type regulatory sequences. These modified sequences can then be reintroduced into the bacterium via homologous recombination into the genome. A non-exhaustive list of preferred promoters that could be used for up-regulation of gene expression includes the promoters *porA*, *porB*, *lbpB*, *tbpB*, *p110*, *lst*, *hpuAB* from *N. meningitidis* or *N. gonorrhoeae*; *ompCD*, *copB*, *lbpB*, *ompE*, *UspA1*; *UspA2*; *TbpB* from *M. Catarrhalis*; *p1*, *p2*, *p4*, *p5*, *p6*, *lpD*, *tbpB*, *D15*, *Hia*, *Hmw1*, *Hmw2* from *H. influenzae*.

In one example, the expression of the gene can be modulated by exchanging its promoter with a stronger promoter (through isolating the upstream sequence of the gene, *in vitro* modification of this sequence, and reintroduction into the genome by homologous

recombination). Upregulated expression can be obtained in both the bacterium as well as in the outer membrane vesicles shed (or made) from the bacterium.

5 In other examples, the described approaches can be used to generate recombinant bacterial strains with improved characteristics for vaccine applications. These can be, but are not limited to, attenuated strains, strains with increased expression of selected antigens, strains with knock-outs (or decreased expression) of genes interfering with the immune response, strains with modulated expression of immunodominant proteins, strains with modulated shedding of outer-membrane vesicles.

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Thus, also provided by the invention is a modified upstream region of the BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 gene, which modified upstream region contains a heterologous regulatory element which alters the expression level of the BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 protein located at the outer membrane. The upstream region according to this aspect of the invention includes the sequence upstream of the BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 gene. The upstream region starts immediately upstream of the BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 gene and continues usually to a position no more than about 1000 bp upstream of the gene from the ATG start codon. In the case of a gene located in a polycistronic sequence (operon) the upstream region can start immediately preceding the gene of interest, or preceding the first gene in the operon. Preferably, a modified upstream region according to this aspect of the invention contains a heterologous promotor at a position between 500 and 700 bp upstream of the ATG.

25

Thus, the invention provides a BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 and BASB071 polypeptide, in a modified bacterial bleb. The invention further provides modified host cells capable of producing the non-live membrane-based bleb vectors. The invention further provides nucleic acid vectors comprising the BASB051,

BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 and BASB071 gene having a modified upstream region containing a heterologous regulatory element.

Further provided by the invention are processes to prepare the host cells and bacterial blebs
5 according to the invention.

Also provided by this invention are compositions, particularly vaccine compositions, and methods comprising the polypeptides and/or polynucleotides of the invention and immunostimulatory DNA sequences, such as those described in Sato, Y. *et al.* Science 273: 352 (1996).

Also, provided by this invention are methods using the described polynucleotide or particular fragments thereof, which have been shown to encode non-variable regions of bacterial cell surface proteins, in polynucleotide constructs used in such genetic immunization experiments in animal models of infection with *Neisseria meningitidis*. Such experiments will be particularly useful for identifying protein epitopes able to provoke a prophylactic or therapeutic immune response. It is believed that this approach will allow for the subsequent preparation of monoclonal antibodies of particular value, derived from the requisite organ of the animal successfully resisting or clearing infection, for the development of prophylactic agents or therapeutic treatments of bacterial infection, particularly *Neisseria meningitidis* infection, in mammals, particularly humans.

The invention also includes a vaccine formulation which comprises an immunogenic recombinant polypeptide and/or polynucleotide of the invention together with a suitable carrier, such as a pharmaceutically acceptable carrier. Since the polypeptides and polynucleotides may be broken down in the stomach, each is preferably administered parenterally, including, for example, administration that is subcutaneous, intramuscular, intravenous, or intradermal. Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteristatic compounds and solutes which render the formulation isotonic with the

5 bodily fluid, preferably the blood, of the individual; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use.

10 The vaccine formulation of the invention may also include adjuvant systems for enhancing the immunogenicity of the formulation. Preferably the adjuvant system raises preferentially a TH1 type of response.

15 An immune response may be broadly distinguished into two extreme categories, being a humoral or cell mediated immune responses (traditionally characterised by antibody and cellular effector mechanisms of protection respectively). These categories of response have been termed TH1-type responses (cell-mediated response), and TH2-type immune responses (humoral response).

20 Extreme TH1-type immune responses may be characterised by the generation of antigen specific, haplotype restricted cytotoxic T lymphocytes, and natural killer cell responses. In mice TH1-type responses are often characterised by the generation of antibodies of the IgG2a subtype, whilst in the human these correspond to IgG1 type antibodies. TH2-type immune responses are characterised by the generation of a broad range of immunoglobulin isotypes including in mice IgG1, IgA, and IgM.

25 It can be considered that the driving force behind the development of these two types of immune responses are cytokines. High levels of TH1-type cytokines tend to favour the induction of cell mediated immune responses to the given antigen, whilst high levels of TH2-type cytokines tend to favour the induction of humoral immune responses to the antigen.

The distinction of TH1 and TH2-type immune responses is not absolute. In reality an individual will support an immune response which is described as being predominantly TH1 or predominantly TH2. However, it is often convenient to consider the families of cytokines in terms of that described in murine CD4 +ve T cell clones by Mosmann and
5 Coffman (*Mosmann, T.R. and Coffman, R.L. (1989) TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annual Review of Immunology, 7, p145-173*). Traditionally, TH1-type responses are associated with the production of the INF- γ and IL-2 cytokines by T-lymphocytes. Other cytokines often directly associated with the induction of TH1-type immune responses are not produced by
10 T-cells, such as IL-12. In contrast, TH2- type responses are associated with the secretion of IL-4, IL-5, IL-6 and IL-13.

It is known that certain vaccine adjuvants are particularly suited to the stimulation of either TH1 or TH2 - type cytokine responses. Traditionally the best indicators of the
15 TH1:TH2 balance of the immune response after a vaccination or infection includes direct measurement of the production of TH1 or TH2 cytokines by T lymphocytes *in vitro* after restimulation with antigen, and/or the measurement of the IgG1:IgG2a ratio of antigen specific antibody responses.

20 Thus, a TH1-type adjuvant is one which preferentially stimulates isolated T-cell populations to produce high levels of TH1-type cytokines when re-stimulated with antigen *in vitro*, and promotes development of both CD8+ cytotoxic T lymphocytes and antigen specific immunoglobulin responses associated with TH1-type isotype.

25 Adjuvants which are capable of preferential stimulation of the TH1 cell response are described in International Patent Application No. WO 94/00153 and WO 95/17209.

3 De-O-acylated monophosphoryl lipid A (3D-MPL) is one such adjuvant. This is known from GB 2220211 (Ribi). Chemically it is a mixture of 3 De-O-acylated
30 monophosphoryl lipid A with 4, 5 or 6 acylated chains and is manufactured by Ribi

Immunochem, Montana. A preferred form of 3 De-O-acylated monophosphoryl lipid A is disclosed in European Patent 0 689 454 B1 (SmithKline Beecham Biologicals SA).

5 Preferably, the particles of 3D-MPL are small enough to be sterile filtered through a 0.22micron membrane (European Patent number 0 689 454).

3D-MPL will be present in the range of 10µg - 100µg preferably 25-50µg per dose wherein the antigen will typically be present in a range 2-50µg per dose.

10 Another preferred adjuvant comprises QS21, an Hplc purified non-toxic fraction derived from the bark of Quillaja Saponaria Molina. Optionally this may be admixed with 3 De-O-acylated monophosphoryl lipid A (3D-MPL), optionally together with a carrier.

The method of production of QS21 is disclosed in US patent No. 5,057,540.

15 Non-reactogenic adjuvant formulations containing QS21 have been described previously (WO 96/33739). Such formulations comprising QS21 and cholesterol have been shown to be successful TH1 stimulating adjuvants when formulated together with an antigen.

20 Further adjuvants which are preferential stimulators of TH1 cell response include immunomodulatory oligonucleotides, for example unmethylated CpG sequences as disclosed in WO 96/02555.

25 Combinations of different TH1 stimulating adjuvants, such as those mentioned hereinabove, are also contemplated as providing an adjuvant which is a preferential stimulator of TH1 cell response. For example, QS21 can be formulated together with 3D-MPL. The ratio of QS21 : 3D-MPL will typically be in the order of 1 : 10 to 10 : 1; preferably 1:5 to 5 : 1 and often substantially 1 : 1. The preferred range for optimal synergy is 2.5 : 1 to 1 : 1 3D-MPL: QS21.

Preferably a carrier is also present in the vaccine composition according to the invention. The carrier may be an oil in water emulsion, or an aluminium salt, such as aluminium phosphate or aluminium hydroxide.

5 A preferred oil-in-water emulsion comprises a metabolisable oil, such as squalene, alpha tocopherol and Tween 80. In a particularly preferred aspect the antigens in the vaccine composition according to the invention are combined with QS21 and 3D-MPL in such an emulsion. Additionally the oil in water emulsion may contain span 85 and/or lecithin and/or tricaprylin.

10

Typically for human administration QS21 and 3D-MPL will be present in a vaccine in the range of 1µg - 200µg, such as 10-100µg, preferably 10µg - 50µg per dose. Typically the oil in water will comprise from 2 to 10% squalene, from 2 to 10% alpha tocopherol and from 0.3 to 3% tween 80. Preferably the ratio of squalene: alpha tocopherol is equal to or less than 1 as this provides a more stable emulsion. Span 85 may also be present at a level of 1%. In some cases it may be advantageous that the vaccines of the present invention will further contain a stabiliser.

15

Non-toxic oil in water emulsions preferably contain a non-toxic oil, e.g. squalene or squalene, an emulsifier, e.g. Tween 80, in an aqueous carrier. The aqueous carrier may be, for example, phosphate buffered saline.

20

A particularly potent adjuvant formulation involving QS21, 3D-MPL and tocopherol in an oil in water emulsion is described in WO 95/17210.

25

The present invention also provides a polyvalent vaccine composition comprising a vaccine formulation of the invention in combination with other antigens, in particular antigens useful for treating cancers, autoimmune diseases and related conditions. Such a polyvalent vaccine composition may include a TH-1 inducing adjuvant as hereinbefore described.

30

Polypeptides, polynucleotides and other compounds of the invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

5 The pharmaceutical compositions may be administered in any effective, convenient manner including, for instance, administration by topical, oral, anal, vaginal, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes among others.

In therapy or as a prophylactic, the active agent may be administered to an individual as an injectable composition, for example as a sterile aqueous dispersion, preferably isotonic.

10

In a further aspect, the present invention provides for pharmaceutical compositions comprising a therapeutically effective amount of a polypeptide and/or polynucleotide, such as the soluble form of a polypeptide and/or polynucleotide of the present invention, agonist or antagonist peptide or small molecule compound, in combination with a pharmaceutically
15 acceptable carrier or excipient. Such carriers include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention. Polypeptides, polynucleotides and other compounds of the present invention may be employed alone or in
20 conjunction with other compounds, such as therapeutic compounds.

The composition will be adapted to the route of administration, for instance by a systemic or an oral route. Preferred forms of systemic administration include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or
25 intraperitoneal, can be used. Alternative means for systemic administration include transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if a polypeptide or other compounds of the present invention can be formulated in an enteric or an encapsulated formulation, oral administration may also be possible. Administration of these compounds may also be topical and/or
30 localized, in the form of salves, pastes, gels, solutions, powders and the like.

Polynucleotide and polypeptide sequences form a valuable information resource with which to determine their 2- and 3-dimensional structures as well as to identify further sequences of similar homology. These approaches are most easily facilitated by storing the sequence in a computer readable medium and then using the stored data in a known macromolecular structure program or to search a sequence database using well known searching tools, such as the GCG program package.

Also provided by the invention are methods for the analysis of character sequences or strings, particularly genetic sequences or encoded protein sequences. Preferred methods of sequence analysis include, for example, methods of sequence homology analysis, such as identity and similarity analysis, DNA, RNA and protein structure analysis, sequence assembly, cladistic analysis, sequence motif analysis, open reading frame determination, nucleic acid base calling, codon usage analysis, nucleic acid base trimming, and sequencing chromatogram peak analysis.

A computer based method is provided for performing homology identification. This method comprises the steps of: providing a first polynucleotide sequence comprising the sequence of a polynucleotide of the invention in a computer readable medium; and comparing said first polynucleotide sequence to at least one second polynucleotide or polypeptide sequence to identify homology.

A computer based method is also provided for performing homology identification, said method comprising the steps of: providing a first polypeptide sequence comprising the sequence of a polypeptide of the invention in a computer readable medium; and comparing said first polypeptide sequence to at least one second polynucleotide or polypeptide sequence to identify homology.

All publications and references, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference in their entirety as if each individual publication or reference were specifically and individually indicated to be

incorporated by reference herein as being fully set forth. Any patent application to which this application claims priority is also incorporated by reference herein in its entirety in the manner described above for publications and references.

5 DEFINITIONS

"Identity," as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as the case may be, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between
10 polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. "Identity" can be readily calculated by known methods, including but not limited to those described in (*Computational Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis*
15 *of Sequence Data*, Part I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heine, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., *SIAM J. Applied Math.*, 48: 1073 (1988). Methods to determine identity are designed to give the largest match between the sequences
20 tested. Moreover, methods to determine identity are codified in publicly available computer programs. Computer program methods to determine identity between two sequences include, but are not limited to, the GAP program in the GCG program package (Devereux, J., et al., *Nucleic Acids Research* 12(1): 387 (1984)), BLASTP, BLASTN (Altschul, S.F. et al., *J. Mol. Biol.* 215: 403-410 (1990), and FASTA(Pearson and Lipman
25 *Proc. Natl. Acad. Sci. USA* 85; 2444-2448 (1988). The BLAST family of programs is publicly available from NCBI and other sources (*BLAST Manual*, Altschul, S., et al., NCBI NLM NIH Bethesda, MD 20894; Altschul, S., et al., *J. Mol. Biol.* 215: 403-410 (1990). The well known Smith Waterman algorithm may also be used to determine identity.

30 Parameters for polypeptide sequence comparison include the following:

Algorithm: Needleman and Wunsch, J. Mol Biol. 48: 443-453 (1970)

Comparison matrix: BLOSSUM62 from Henikoff and Henikoff,

Proc. Natl. Acad. Sci. USA. 89:10915-10919 (1992)

Gap Penalty: 8

5 Gap Length Penalty: 2

A program useful with these parameters is publicly available as the "gap" program from Genetics Computer Group, Madison WI. The aforementioned parameters are the default parameters for peptide comparisons (along with no penalty for end gaps).

10 Parameters for polynucleotide comparison include the following:

Algorithm: Needleman and Wunsch, J. Mol Biol. 48: 443-453 (1970)

Comparison matrix: matches = +10, mismatch = 0

Gap Penalty: 50

Gap Length Penalty: 3

15 Available as: The "gap" program from Genetics Computer Group, Madison WI. These are the default parameters for nucleic acid comparisons.

A preferred meaning for "identity" for polynucleotides and polypeptides, as the case may be, are provided in (1) and (2) below.

20

(1) Polynucleotide embodiments further include an isolated polynucleotide comprising a polynucleotide sequence having at least a 50, 60, 70, 80, 85, 90, 95, 97 or 100% identity to the reference sequence of SEQ ID NO:1, wherein said polynucleotide sequence may be identical to the reference sequence of SEQ ID NO:1 or may include up to a certain integer

25 number of nucleotide alterations as compared to the reference sequence, wherein said alterations are selected from the group consisting of at least one nucleotide deletion, substitution, including transition and transversion, or insertion, and wherein said alterations may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among the
30 nucleotides in the reference sequence or in one or more contiguous groups within the

reference sequence, and wherein said number of nucleotide alterations is determined by multiplying the total number of nucleotides in SEQ ID NO:1 by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of nucleotides in SEQ ID NO:1, or:

5

$$n_n \leq x_n - (x_n \bullet y),$$

wherein n_n is the number of nucleotide alterations, x_n is the total number of nucleotides in SEQ ID NO:1, y is 0.50 for 50%, 0.60 for 60%, 0.70 for 70%, 0.80 for 80%, 0.85 for 85%,
 10 0.90 for 90%, 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and \bullet is the symbol for the multiplication operator, and wherein any non-integer product of x_n and y is rounded down to the nearest integer prior to subtracting it from x_n . Alterations of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:2 may create nonsense, missense or frameshift mutations in this coding sequence and thereby alter the polypeptide encoded by
 15 the polynucleotide following such alterations.

By way of example, a polynucleotide sequence of the present invention may be identical to the reference sequence of SEQ ID NO:1, that is it may be 100% identical, or it may include up to a certain integer number of nucleic acid alterations as compared to the reference
 20 sequence such that the percent identity is less than 100% identity. Such alterations are selected from the group consisting of at least one nucleic acid deletion, substitution, including transition and transversion, or insertion, and wherein said alterations may occur at the 5' or 3' terminal positions of the reference polynucleotide sequence or anywhere between those terminal positions, interspersed either individually among the nucleic acids
 25 in the reference sequence or in one or more contiguous groups within the reference sequence. The number of nucleic acid alterations for a given percent identity is determined by multiplying the total number of nucleic acids in SEQ ID NO:1 by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of nucleic acids in SEQ ID NO:1, or:

30

By way of example, a polypeptide sequence of the present invention may be identical to the reference sequence of SEQ ID NO:2, that is it may be 100% identical, or it may include up to a certain integer number of amino acid alterations as compared to the reference sequence such that the percent identity is less than 100% identity. Such alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence. The number of amino acid alterations for a given % identity is determined by multiplying the total number of amino acids in SEQ ID NO:2 by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of amino acids in SEQ ID NO:2, or:

$$n_a \leq x_a - (x_a \bullet y),$$

wherein n_a is the number of amino acid alterations, x_a is the total number of amino acids in SEQ ID NO:2, y is, for instance 0.70 for 70%, 0.80 for 80%, 0.85 for 85% etc., and \bullet is the symbol for the multiplication operator, and wherein any non-integer product of x_a and y is rounded down to the nearest integer prior to subtracting it from x_a .

"Individual(s)," when used herein with reference to an organism, means a multicellular eukaryote, including, but not limited to a metazoan, a mammal, an ovid, a bovid, a simian, a primate, and a human.

"Isolated" means altered "by the hand of man" from its natural state, *i.e.*, if it occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living organism is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural

state is "isolated", as the term is employed herein. Moreover, a polynucleotide or polypeptide that is introduced into an organism by transformation, genetic manipulation or by any other recombinant method is "isolated" even if it is still present in said organism, which organism may be living or non-living.

5

"Polynucleotide(s)" generally refers to any polyribonucleotide or polydeoxyribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA including single and double-stranded regions.

10 "Variant" refers to a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes
15 may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and
20 reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides
25 and polypeptides may be made by mutagenesis techniques or by direct synthesis.

"Disease(s)" means any disease caused by or related to infection by a bacteria, including , for example, upper respiratory tract infection, invasive bacterial diseases, such as bacteremia and meningitis.

EXAMPLES

The examples below are carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. The examples
5 are illustrative, but do not limit the invention.

Example 1

The BASB051 gene in *N.meningitidis* strain ATCC 13090.

10

The BASB051 gene of *N. meningitidis* strain ATCC 13090 is shown in SEQ ID NO:1. The translation of the BASB051 polynucleotide sequence, shown in SEQ ID NO:2, shows significant similarity to *Neisseria gonorrhoeae* ComL lipoprotein. The BASB051 polypeptide contains a leader sequence characteristic of a lipoprotein signal sequence.

15

Example 2

The BASB057 gene in *N.meningitidis* strain ATCC 13090.

20 The BASB057 gene of *N. meningitidis* strain ATCC 13090 is shown in SEQ ID NO:3. The translation of the BASB057 polynucleotide sequence, shown in SEQ ID NO:4, shows significant similarity to *Neisseria gonorrhoeae* MtrE outer membrane lipoprotein. The BASB057 polypeptide contains a leader sequence characteristic of a lipoprotein signal sequence.

25

Example 3

The BASB060 gene in *N.meningitidis* strain ATCC 13090.

The BASB060 gene of *N. meningitidis* strain ATCC 13090 is shown in SEQ ID NO:5.
The translation of the BASB060 polynucleotide sequence, shown in SEQ ID NO:6,
shows no significant similarity to any known protein. However, the BASB060
polypeptide contains a leader sequence which is characteristic of a lipoprotein signal
sequence and has the characteristics of an outer membrane lipoprotein.

Example 4

The BASB061 gene in *N.meningitidis* strain ATCC 13090.

The BASB061 gene of *N. meningitidis* strain ATCC 13090 is shown in SEQ ID NO:7.
The translation of the BASB061 polynucleotide sequence, shown in SEQ ID NO:8,
shows significant similarity to *Neisseria meningitidis* mlp gene product. The BASB061
polypeptide contains a leader sequence which is characteristic of a lipoprotein signal
sequence and has the characteristics of an outer membrane lipoprotein.

Example 5

The BASB063 gene in *N.meningitidis* strain ATCC 13090.

The BASB063 gene of *N. meningitidis* strain ATCC 13090 is shown in SEQ ID NO:9.
The translation of the BASB063 polynucleotide sequence, shown in SEQ ID NO:10,
shows significant similarity to any known protein. However, the BASB063 polypeptide
does contain a leader sequence which is characteristic of a lipoprotein signal sequence
and has the characteristics of an outer membrane lipoprotein.

Example 6

The BASB065 gene in *N.meningitidis* strain ATCC 13090.

The BASB065 gene of *N. meningitidis* strain ATCC 13090 is shown in SEQ ID NO:11. The translation of the BASB065 polynucleotide sequence, shown in SEQ ID NO:12, shows significant similarity to any known protein. However, the BASB065 polypeptide does contain a leader sequence which is characteristic of a lipoprotein signal sequence and has the characteristics of an outer membrane lipoprotein.

Example 7

The BASB066 gene in *N.meningitidis* strain ATCC 13090.

The BASB066 gene of *N. meningitidis* strain ATCC 13090 is shown in SEQ ID NO:13. The translation of the BASB066 polynucleotide sequence, shown in SEQ ID NO:14, shows significant similarity to *Neisseria meningitidis* CtrA protein. The BASB066 polypeptide contains a leader sequence which has the characteristics of a lipoprotein signal sequence and has the characteristics of a protein located in the outer membrane.

Example 8

The BASB071 gene in *N.meningitidis* strain ATCC 13090.

The BASB071 gene of *N. meningitidis* strain ATCC 13090 is shown in SEQ ID NO:15. The translation of the BASB071 polynucleotide sequence, shown in SEQ ID NO:16, shows significant similarity to *Neisseria gonorrhoeae* HisJ protein. The BASB071 polypeptide is contains a leader sequence which has the characteristics of a lipoprotein signal sequence.

Polynucleotide and Polypeptide Sequences

SEQ ID NO:1

Neisseria meningitidis BASB051 polynucleotide sequence from strain ATCC 13090

5 ATGAAAAAATTCTTTTAACGGTTTCATTAGGTTTGGCACTGAGTGCCTGTGCCACTCAA
GGTACGGTCGATAAAGATGCTCAGATTACCCAAGATTGGAGTGTGGAGAAGCTCTATGCC
GAAGCCCAGGACGAATTGAACAGCAGCAATTATACGCGGGCTGTCAAGTTATACGAAATC
TTGGAATCGCGCTTCCCCACCAGCCGCCATGCCCCGGAATCCCAACTGGATACCGCATAC
GCCTATTATAAAGACGATGAAAAAGACAAGGCTCTGGCGGCAATCGAACGCTTCCGCCGC
10 CTCCATCCGCAGCATCCGAATATGGATTACGCGCTGTATCTGCGCGGCTTGGTGTCTGTTT
AACGAAGACCAGTCCTTCTTGAACAAACTGGCCTCGCAAGACTGGTCCGACCGCGACCCG
AAAGCCAACCGCGAAGTAACCCAGGCGTTTGCAGCACTCGTCCAACGCTTCCCCAACAGC
AAATACGCGCGCGATGCGACCGCACGCATGGTCAAACCTGGTTCGATGCACTGGGCGGCAAT
GAAATGTCTGGTGGCGCGCTACTACATGAAACGCGCGCATATATCGCCGCCGCCAACCGC
15 GCCCCAAAAATTATCGGCAGCTACCAAAATACACGCTATGTCTGAAGAATCGCTCGCCATC
TTGGAACCTTGCCATACCAAAACTCGGCAAAACACAGCTTGCCGCCGATACGCGCGCGTG
TTGGAACCAACTTCCCCGAAAAGCCGTTTTTGTACGCACGCTTGGCAGCCCGACGATATG
CCTTGGTGGCGTTACTGGCATTA

20 SEQ ID NO:2

Neisseria meningitidis BASB051 polypeptide sequence deduced from the polynucleotide sequence of SEQ ID NO:1

25 MKKILLTVSLGLALSACATQGTVDKDAQITQDWSVEKLYAEAQDELNSSNYTRAVKLYEI
LESRFPTSRHARQSLDTAYAYYKDDEKDALAAIERFRRLHPQHNPMDYALYLRGLVLF
NEDQSFLNKLASQDWSDRDPKANREVTQAFBELVQRFNSKYAADATARMVKLVLDALGGN
EMSVARYYMKRGAYIAAANRAQKIIGSYQNTRYVEESLAILELAYQKLGKPQLAADTRRV
LETNFPKSPFLTHAWQPDDMPWRYWH

SEQ ID NO:3

30 *Neisseria meningitidis* BASB057 polynucleotide sequence from strain ATCC 13090

ATGGATACTACATTGAAAACACCTTGACTTCTGTTGCAGCAGCCTTCGCATTATCCGCC
TGCACCATGATTCCCCAATACGAGCAGCCCAAAGTCGAAGTTGCCGAAACGTTTAAAAAC
GATACCGCCGACAGCGGCATCCGTGCGGTGCGATTAGGTTGGCATGACTATTTTGCCGAC
CCGCGCCTGCAAAAGCTGATCGACATCGCACTCGAGCGCAATACCAGTTTGCGTACCGCC
35 GTATTGAACAGCGAAATCTACCGCAAAACAATACATGATTGAGCGCAACAACCTCCTGCCC
ACGCTTGCCGCCAATGCGAACGGCTCGCGCCAAGGCAGCTTGAGCGGCGGCAATGTGAGC
AGCAGCTACAATGTGCGACTGGGTGCGGCATCTTACGAACTCGACCTGTTGCGACGCGTC
CGCAGCAGCAGCGAAGCAGCACTGCAAGGCTATTTTGCAAGTGTGCGCAACCGCGATGCG
GCACATTTGAGCCTGATTGCCACCGTTGCCAAAGCCTATTTCAACGAACGTTATGCCGAA
40 GAAGCGATGTCTTTGGCGCAGCGTGTGTTTGAACACGCGCGAGGAAACCTACAAGCTGTCC
GAATTACGTTACAAGGCAGGCGTGATTTCCGCCGTGCGCCTACGTGAGCAGGAAGCCCTG
ATCGAATCTGCCAAAGCCGATTATGCCCATGCCGCGCGCAGCCGCAACAGGCGCGCAAT
GCCTTGCGAACCTTGATTAACCAACCGATACCCGAAGACCTGCCTGCCGCTTTGCCGCTG
GACAAGCAGTTTTTTGTTGAAAACTGCCGCGCGGTTTGAGTTCCGAAGTATTGCTCGAC
45 CGTCCCGATATCCGTGCTGCCGAACACGCGCTCAAACAGGCAAACGCCAATATCGGTGCG
GCACGCGCCGCTTTTTCCCATCCATCCGCCTGACCGGAACCGTCGGTACGGGTTCTGCC

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GAATTGGGTGGGTTGTTCAAAAAGCGGCACGGGCGTTTGGTCGTTTCGCGCCGTCTATTACC
CTGCCGATTTTTACCTGGGGTACGAACAAAGCCAACCTTGATGTAGCCAAGCTGCGCCAA
CAGGCACAAATCGTTGCCTATGAAGCCGCGTCCAATCCGCATTTCAAGACGTGGCAAAC
GCATTGGCGGCGCGGAGCAGCTGGATAAAGCCTATGACGCTTTAAGCAAACAAAGCCGC
5 GCCTCTAAAGAGGCGTTGCGCTTGGTCGGCCTGCGTTACAAGCACGGCGTATCCGGCGCG
CTCGACTTGCTCGATGCGGAACGCAGCAGCTATGCGGCGGAGGGTGC GGCTTTGTCTGGCA
CAACTGACCCGCGCCGAAAACCTTGCCGATTTGTACAAGGCACTCGGCGGCGGATTGAAA
CGGGATACCCAAACCGACAAATAA

10 SEQ ID NO:4

Neisseria meningitidis BASB057 polypeptide sequence deduced from the polynucleotide sequence of SEQ ID NO:3

MDTTLKTTLTSVAAAFALSACTMIPQYEQPKVEVAETFKNDTADSGIRAVDLGWHDYFAD
PRLQKLIDIALERNTSLRTAVLNSEIYRKQYMIERNLLPTLAANANGSRQGSLSGGNVS
15 SSYNVGLGAASYELDLFGRVRSSEALQGYFASVANRDAHLSLIATVAKAYFNERYAE
EAMSLAQRVLKTRREETYKLSELRYKAGVISAVALRQQEALIESAKADYAHAARSREQARN
ALATLINQPIPEDLPAGLPLDKQFFVEKLPAGLSSEVLLDRPDIRAAEHALKQANANIGA
ARAAFFPSIRLTGTGTGSAELGGLFKSGTGVWSFAPSITLPIFTWGTNKANLDVAKLRQ
QAQIVAYEAAVQSAFQDVANALAAREQLDKAYDALSKQSRASKEALRLVGLRYKHGVSGA
20 LDLLDAERSSSYAEGAALSAQLTRAENLADLYKALGGGLKRDQTQDK

SEQ ID NO:5

Neisseria meningitidis BASB060 polynucleotide sequence from strain ATCC 13090

25 ATGAAAAAACTTCTAATGATAACCCCTACCGGTATGCTTGCAGCTTGTGCAACAGGTGTC
AATGTCGGCCGCTTGATGGTTGAAATGCCGCAGGGAGAACGTTCTGTCTGTTGTGAGGTT
CCCGCGACAAATAACCCGCTTTCCGATACGGTAGCTGTGCGAATGATTAAACATCCGGT
TCGCCTTCGGCATCAAATATGATTGAAATGCTCGGCGCGGACAATATCAACGTGCGGCGTG
GTGGGAAGCAGCCAAATGCTTAATAAGGCGACCGCACTTTATTCCTTAAACCATGCAAAG
30 AAAGTCGGAAATAATGTCTAGTGTATATGATGGGCGACAGCGAAAGTGACAAGGCCGAT
TTGGAAAACGCGGCAAATGCCAAAAATATCAAATTGCATTATTTCTTTAACCAAAAATAA

SEQ ID NO:6

35 *Neisseria meningitidis* BASB060 polypeptide sequence deduced from the polynucleotide sequence of SEQ ID NO:5

MKKLLMITLTGMLAACATGVNVGRLMVEMPQERSVVVQVPATNNPLSDTVAVGMIKTSG
SPSASNMIEMLGADNINVGVSQMLNKATALYSLNHAKKVGNNVSVYMMGDSESDKAD
LENAANAKNIKLHYFFNQK

40 SEQ ID NO:7

Neisseria meningitidis BASB061 polynucleotide sequence from strain ATCC 13090

ATGAAAATCAAACAAATCGTCAAACCGGGCTTGGCAGTATTGGCGGCGGGCGTTCTGTCT
GCCTGCGCAACCAAAAGCAACGTCAAAGCCGACGGAACGACCGACAATCCGGTTTCCCG
AAACCTATTCCGTAACGCTCGACAACAATCGCGGTACATTCCCGACCTATGACGAATTG

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5 GACTTGATGCGTCCCGGTCTGACCAAAGACGACATCTACAAAATCCTGGGTCGTCCGCAT
TACGACGAAGGTATGTACGGCGTGC CGGAATGGGATTATCTGTTCCACTTCCACACCCCG
GGCGTAGGCATCGACCCTGAAAACACTTCCGGCGTAGAAGGCATTACCACCTGTCAATAC
AAAATTATTTTCGATAAAGACAAATTTGCCCGCAGCTTCTACTGGAACCCCGTCTTCCCG
AAAGATGCCGCTGTCCGCCGCCCGCACCCAAAGCCGAGCCGCAgTCATCATCCGCGAA
ATCGTGCCCGCCAAAcCCAAACGCATCCGCCAATAA

SEQ ID NO:8

10 *Neisseria meningitidis* BASB061 polypeptide sequence deduced from the polynucleotide
sequence of SEQ ID NO:7

MKIKQIVKPGLAVLAAGVLSACATKSNVKADGTTDNPVFPKPYSVTLDNNRGTFPTYDEL
DLMRPGLTKDDIYKILGRPHYDEGMYGVREWDYLFHFHTPGVGIDPENTSGVEGITTCQY
KIIFDKDKFARSFYWNPVFPKDAACPPPAKPAEPQVIIREIVPAKPKRIRQ

15 SEQ ID NO:9

Neisseria meningitidis BASB063 polynucleotide sequence from strain ATCC 13090

20 ATGAGACCATATGCTACTACCATTTATCAACTTTTTATTTGTTTATTGGGAGTGTTTTT
ACTATGACCTCATGTGAACCTGTGAATGAAAAGACAGATCAAAAAGCAGTAAGTGCGCAA
CAGGCTAAAGAACAACACAGTTTCAACAATCCCGAGCCAATGACAGGATTTGAACATACG
GTTACATTTGATTTTTCAGGGCACCAAAATGGTTATCCCCTATGGCTATCTTGACGGTAT
ACGCAAGACAATGCCACAAAATGGCTTTCCGACACGCCCCGGGCAGGATGCTTACTCCATT
AATTTGATAGAGATTAGCGTCTATTACAAAAAACCGACCAAGGCTGGGTTCTTGAGCCA
TACAACCAGCAAAACAAGCACACTTTATCCAATTTCTACGCGACGGTTTGGATAGCGTG
25 GACGATATTGTTATCCGAAAAGATGCGGTAGTTTAAGTACGACTATGGGAGAAAGATTG
CTTACTTACGGGGTTAAAAAATGCCATCTGCCTATCCTGAATACGAGGCTTATGAAGAT
AAAAGACATATTCCTGAAAATCCATATTTTCATGAATTTTACTATATTA AAAAAGGAGAA
AATCCGGCGATTATTACTCATCGGAATAATCGAATAAACCAAACCTGAAGAAGATAGTTAT
AGCACTAGCGTAGGTTCTGTATTAACGGTTTTCACGGTACAGTATTACCCGTTTATTTCGG
30 GAAAAGCAGCAGCTCACACAGCAGGAGTTGGTAGGTTATACCAACAAGTAGAGCAATTG
GTACAGAGTTTTGTAAACAATTCAAATAAAAAATAA

SEQ ID NO:10

Neisseria meningitidis BASB063 polypeptide sequence deduced from the polynucleotide
sequence of SEQ ID NO:9

35 MRPYATTIYQLFILFIGSVFTMTSCEPVNEKTDQKAVSAQQAQKEQTSFNNPEPMTGFEHT
VTFDFQGTMVIPYGYLARYTQDNATKWLS DTPGQDAYSINLIEISVYYKKT DQGWVLEP
YNQONKAHFIQFLRDGLDSVDDIVIRKDACSLSTTMGERLLTYGVKKMPSAYPEYEAYED
KRHIPENPYFHEFYIKKGENPAIIITHRNNRINQTEEDSYSTSVGSCINGFTVQYYPFIR
EKQQLTQQELVGYHQQVEQLVQSFVNNSNKK

40

SEQ ID NO:11

Neisseria meningitidis BASB065 polynucleotide sequence from strain ATCC 13090

ATGAAGACCAAATTACCGCTTTTTATCATTTGGCTGTCCGTATCCGCCGCTGTTCTTCC

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5 CCTGTTTCCCGCAATATTTCAGGATATGCGGCCCGAACCGCAGGCAGAGGCAGGTAGTTTCG
GACGCTATTCCCTATCCCGTTCCCACTCTGCAAGACCGTTTGGATTATCTGGAAGGCACA
CTCGTCCGCCTGTGCAACGAAGTGGAACCTTAAACGGCAAAGTCAAAGCACTGGAGCAT
GCGAAACACACCCCTTCCGGTAGGGCATACTCCAAAACTCGACGACCGCAAGTTGAAA
10 GAGCATTACCTCAATACCGAAGGCGGCAGCGCATCCGCACATACCGTCGAAACCGCACAA
AACCTCTACAATCAGGCACTCAAACACTATAAAAGCGGCAGGTTTTCTGCCGCAGCCGCC
CTGTTGAAAGGCGCGGACGGAGGCGACGGCGGCAGCATCGCGCAACGCAGTATGTACCTG
TTGCTGCAAAGCAGGGCGCGTATGGGCAACTGCGAATCCGTCATCGAAATCGGAGGGCGT
TACGCCAACCGTTTCAAAGACAGCCCAACCGCGCCCGAAGCCATGTTCAAATCGGCGAA
15 TGCCAATACAGGTTGCAGCAGAAAGACATTGCAAGGGCAACTTGGCGCAGCCTGATACAG
GCTTACCCGAGCAGCCCGCGGCAAAACGCGCCCGCAGCCGTACGCAAACGATAG

SEQ ID NO:12

15 *Neisseria meningitidis* BASB065 polypeptide sequence deduced from the polynucleotide
sequence of SEQ ID NO:11

20 MKTKLPLFIIWLSVSAACSSPVSRNIQDMRPEPQAEAGSSDAIPYPVPTLQDRLDYLEGT
LVRLSNEVETLNGKVKALEHAKTHPSGRAYVQKLDRLKEHYLNTEGGSASAHTVETAQ
NLYNQALKHYKSGRFSAAAALLKGADGGDGSIAQRSMYLLQLSRARMGNCESVIEIGGR
YANRFKDSPTAPEAMFKIGECQYRLQKDIARATWRSLIQAYPSSPAAKRAAAVRKR

SEQ ID NO:13

Neisseria meningitidis BASB066 polynucleotide sequence from strain ATCC 13090

25 GTGTTTAAAGTGAAATTTTATATTTCGTCACGCAGTATTATTATTGTGTGGAAGTTTAATT
GTAGGATGCTCTGCGATTCTTCATCAGGCCCCAGCGCAAAAAAATTGTCTCTTTAGGG
CAACAATCTGAAGTTCAAATTCCTGAAGTGGAGCTGATTGATGTGAATCATACGGTTGCT
CAGTTATTATATAAGGCTCAGATAAATCAGTCATTCACTCAGTTTGGCGATGGTTATGCT
TCGGCTGGTACGCTAAATATTGGTGATGTATTGGATATTATGATTTGGGAAGCGCCCGC
GCAGTATTGTTGGTGGTGGCCTTTCTTCGATGGGCTCGGGTAGTGCGCATCAAATAAG
30 TTGCCAGAGCAGTTGGTCACGGCACGTGGTACGGTTTCTGTGCCGTTTGTGGCGATATT
TCGGTGGTTCGTTAAACGCCTGGTCAGGTTTCAGGAAATTATTAAAGGCCGCTGAAAAAA
ATGGCCAATCAGCCACAAGTGATGGTGCGTTTGGTGCAGAATAATGCGGCGAATGTGTCTG
GTGATTCGTGCTGGGAATAGTGTGCGTATGCCGCTGACGGCAGCCGGTGAGCGTGTGTTG
GATGCGGTGGCTGCGGTAGGTGGTTCAACGGCAAATGTGCAGGATACGAATGTGCAGCTG
ACACGTGGCAATGTAGTACGAACGTGTGCCTTGGAAGATTTAGTTGCAAATCCGCGACAA
35 AATATTTTGTGCTGCGTTCGCGGTGATGTGGTTACCATGATTACCAATCCCTATACCTTTACG
TCTATGGGTGCGGTGGGGAGAACACAAGAAATCGGTTTTTTCAGCCAGAGGCTTATCGCTT
TCTGAAGCCATTGGCCGTATGGGCGGTTTGCAAGATCGCCGTTCTGATGCGCGTGGTGTG
TTTGTGTTCCGCTATACGCCATTGGTGGAATTGCCGGCAGAACGTGAGGATAAATGGATT
GCTCAAGGTTATGGCAGTGAGGCAGAGATTCCAACGGTATATCGTGTGAATATGGCTGAT
40 GCGCATTGCTATTTTCTATGCAGCGCTTTCCTGTGAAGAATAAAGATGTATTGTATGTG
TCGAATGCGCCGTTGGCTGAAGTGCAGAAATCTTGTGCTTTGTGTTCTCGCCGGTTACC
AGTGGCGCGAACAGTATTAATAATTTAACTAATTAA

SEQ ID NO:14

45 *Neisseria meningitidis* BASB066 polypeptide sequence deduced from the polynucleotide
sequence of SEQ ID NO:13

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MFKVKFYIRHAVLLLCGSLIVGCSAIPSSGSPSAKKIVSLGQQSEVQIPEVELIDVNHTVA
QLLYKAQINQSFTQFGDGYASAGTLNIGDVLDIMIWEAPPAVLFGGGLSSMGSASHQTK
LPEQLVTARGTVSVPFVGDISVVGKTPGQVQEI IKGRLKKMANQPQVMVRLVQNNAA NVS
VIRAGNSVRMPLTAAGERVLDAAVAAGGSTANVQDTNVQLTRGNVVRTVALEDLVANPRQ
5 NILLRRGDVVTMITNPYTFTSMGAVGRTQEIGFSARGLSLSEAIGRMGGLQDRRSDARGV
FVFRYTPLVLPALPAERQDKWIAQGYGSEAEIPTVYRVNMADAHSLSMQRFPVKNKDVLYV
SNAPLAEVQKFLSFVFSVPTSGANSINNLTN

SEQ ID NO:15

10 *Neisseria meningitidis* BASB071 polynucleotide sequence from strain ATCC 13090

ATGAATATGAAAAAATGGATTGCCGCCGCCCTTGCCCTGTTCCGCGCTCGCGCTGTCTGCC
TGCGGCGGT CAGGGCAAAGATGCCGCCGCCGCCGCCGCAAACCCCGACAAAGTGTACCGC
GTGGCTTCCAACGCCGAGTTTGCCCCCTTTGAATCTTTAGACTCGAAAGGCAATGTTGAA
GGTTTCGATGTGGATTTGATGAACGCGATGGCGAAGGCGGGCAATTTTAAATCGAATTC
15 AAACACCAGCCGTGGGACAGCCTTTTCCCCGCCCTTGAACAACGGCGATGCGGACGTTGTG
ATGTCGGGCGTAACCATTAACGACGACCGCAAACAGTCTATGGACTTCAGCGACCCGTAT
TTTGAAATCACCCTCGTCCTCGTTCCGAAAGGCAAAAAATATCTTCTTCCGAAGAT
TTGAAAAACATGAACAAAGTCGGCGTGGTAACCGGCTACACGGGCGATTTCTCCGTATCC
AAACTCTTGGGCAACGACAACCCGAAAATCGCGCGCTTTGAAAACGTTCCCTGATTATC
20 AAAGAACTGGAAAACGGCGGCTTGGATTCCGTGGTCAGCGACAGCGCAGTCATCGCCAAT
TATGTGAAAAACAATCCGACCAAAGGGATGGACTTCGTTACCCGTGCCGACTTCACCACC
GAACACTACGGCATCGCGGTACGCAAAGGCGACGAAGCAACCGTCAAATGCTGAACGAT
GCGTTGAAAAAAGTACGCGAAAGCGGCGAATACGACAAAATCTACGCCAAATATTTTGCA
AAAGAAGACGGACAGGCCGCAAAATAA

25

SEQ ID NO:16

Neisseria meningitidis BASB071 polypeptide sequence deduced from the polynucleotide
sequence of SEQ ID NO:15

30 MNMKKWIAAALACSALALSACGGQKGDAAPAAPNDKVYRVASNAEFAPFESLDSKGNVE
GFDVLDLMNAMAKAGNFKIEFKHPWDSLFPALNNGDADVMSGVTITDDRQSMDFSDPY
FEITQVVLVPKGKKISSSEDLKNMKNKGVVVTGYTGDFSVSKLLGNDNPKIARFENVPLII
KELENGGLDSVSDSAVIANYVKNNPTKGMDFVTLPDFTTEHYGIAVRKGDEATVKMLND
ALKKVRESGEYDKIYAKYFAKEDGQAAK

Deposited materials

A deposit containing a *Neisseria meningitidis* Serogroup B strain has been deposited with the American Type Culture Collection (herein "ATCC") on June 22, 1997 and assigned deposit number 13090. The deposit was described as *Neisseria meningitidis* (Albrecht and Ghon) and is a freeze-dried, 1.5-2.9 kb insert library constructed from *N. meningitidis* isolate. The deposit is described in Int. Bull. Bacteriol. Nomencl. Taxon. 8: 1-15 (1958).

The *Neisseria meningitidis* strain deposit is referred to herein as "the deposited strain" or as "the DNA of the deposited strain."

10

The deposited strain contains the full-length BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 and BASB071 genes. The sequence of the polynucleotides contained in the deposited strain, as well as the amino acid sequence of any polypeptide encoded thereby, are controlling in the event of any conflict with any description of sequences herein.

15

The deposit of the deposited strain has been made under the terms of the Budapest Treaty on the International Recognition of the Deposit of Micro-organisms for Purposes of Patent Procedure. The strain will be irrevocably and without restriction or condition released to the public upon the issuance of a patent. The deposited strain is provided merely as convenience to those of skill in the art and is not an admission that a deposit is required for enablement, such as that required under 35 U.S.C. §112.

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Applicant's or agent's file reference	KP/BM45348	International application No.
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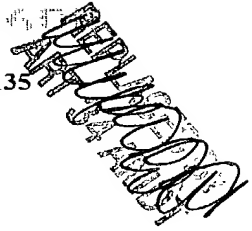
**INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL**

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page <u>110</u> , line <u>2-22</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution AMERICAN TYPE CULTURE COLLECTION	
Address of depositary institution (including postal code and country) 10801 UNIVERSITY BLVD, MANASSAS, VIRGINIA 20110-2209, UNITED STATES OF AMERICA	
Date of deposit 22 June 1997 (22.06.97)	Accession Number 13090
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
In respect of those designations where a European Patent is sought, a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European Patent or until the date on which the application has been refused or withdrawn, only by issue of such a sample to an expert nominated by the person requesting the sample.	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g. "Accession Number of Deposit")	

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CLAIMS

1. An isolated polypeptide comprising an amino acid sequence which has at least 85% identity to an amino acid sequence selected from the group consisting of: SEQ ID NO:2,
5 SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16.
2. An isolated polypeptide as claimed in claim 1 in which the amino acid sequence has at least 95% identity to the amino acid sequence selected from the group consisting of: SEQ
10 ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16.
3. The polypeptide as claimed in claim 1 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8,
15 SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16.
4. An isolated polypeptide of : SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14 or SEQ ID NO:16.
- 20 5. An immunogenic fragment of the polypeptide as claimed in any one of claims 1 to 4 in which the immunogenic activity of said immunogenic fragment is substantially the same as that of the polypeptide of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14 or SEQ ID NO:16.
- 25 6. An isolated polynucleotide comprising a nucleotide sequence encoding a polypeptide that has at least 85% identity to an amino acid sequence of SEQ ID NO:2,4,6,8,10,12,14 or 16 over the entire length of SEQ ID NO:2,4,6,8,10,12,14 or 16 respectively; or a nucleotide sequence complementary to said isolated polynucleotide.

21. A method of diagnosing a *Neisseria meningitidis* infection, comprising identifying a polypeptide as claimed in any one of claims 1 to 5, or an antibody that is immunospecific for said polypeptide, present within a biological sample from an animal suspected of having
5 such an infection.
22. Use of a composition comprising an immunologically effective amount of a polypeptide as claimed in any one of claims 1 to 5 in the preparation of a medicament for use in generating an immune response in an animal.
10
23. Use of a composition comprising an immunologically effective amount of a polynucleotide as claimed in any one of claims 6 to 12 in the preparation of a medicament for use in generating an immune response in an animal.
- 15 24. A therapeutic composition useful in treating humans with *Neisseria meningitidis* disease comprising at least one antibody directed against the polypeptide of claims 1 to 5 and a suitable pharmaceutical carrier.

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C12N 15/31, C07K 14/22, A61K 39/095, C07K 16/12, G01N 33/569		A3	(11) International Publication Number: WO 00/42191 (43) International Publication Date: 20 July 2000 (20.07.00)																																				
(21) International Application Number: PCT/EP00/00135 (22) International Filing Date: 10 January 2000 (10.01.00) (30) Priority Data: <table border="0"><tr><td>9900952.4</td><td>15 January 1999 (15.01.99)</td><td>GB</td></tr><tr><td>9900838.5</td><td>15 January 1999 (15.01.99)</td><td>GB</td></tr><tr><td>9901945.7</td><td>28 January 1999 (28.01.99)</td><td>GB</td></tr><tr><td>9901948.1</td><td>28 January 1999 (28.01.99)</td><td>GB</td></tr><tr><td>9902078.6</td><td>29 January 1999 (29.01.99)</td><td>GB</td></tr><tr><td>9902088.5</td><td>29 January 1999 (29.01.99)</td><td>GB</td></tr><tr><td>9902074.5</td><td>29 January 1999 (29.01.99)</td><td>GB</td></tr><tr><td>9902879.7</td><td>9 February 1999 (09.02.99)</td><td>GB</td></tr><tr><td>9902936.5</td><td>10 February 1999 (10.02.99)</td><td>GB</td></tr><tr><td>9903978.6</td><td>20 February 1999 (20.02.99)</td><td>GB</td></tr><tr><td>9904133.7</td><td>23 February 1999 (23.02.99)</td><td>GB</td></tr><tr><td>9904404.2</td><td>25 February 1999 (25.02.99)</td><td>GB</td></tr></table> (71) Applicant (for all designated States except US): SMITHK- LINE BEECHAM BIOLOGICALS S.A. [BE/BE]; Rue de l'Institut 89, B-1330 Rixensart (BE). (72) Inventors; and (75) Inventors/Applicants (for US only): RUELLE, Jean-Louis [BE/BE]; SmithKline Beecham Biologicals S.A., Rue de l'Institut 89, B-1330 Rixensart (BE). THONNARD, Joelle			9900952.4	15 January 1999 (15.01.99)	GB	9900838.5	15 January 1999 (15.01.99)	GB	9901945.7	28 January 1999 (28.01.99)	GB	9901948.1	28 January 1999 (28.01.99)	GB	9902078.6	29 January 1999 (29.01.99)	GB	9902088.5	29 January 1999 (29.01.99)	GB	9902074.5	29 January 1999 (29.01.99)	GB	9902879.7	9 February 1999 (09.02.99)	GB	9902936.5	10 February 1999 (10.02.99)	GB	9903978.6	20 February 1999 (20.02.99)	GB	9904133.7	23 February 1999 (23.02.99)	GB	9904404.2	25 February 1999 (25.02.99)	GB	[BE/BE]; SmithKline Beecham Biologicals S.A., Rue de l'Institut 89, B-1330 Rixensart (BE). (74) Agent: PRIVETT, Kathryn, Louise; SmithKline Beecham, Two New Horizons Court, Brentford, Middlesex TW8 9EP (GB). (81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report</i> (88) Date of publication of the international search report: 16 November 2000 (16.11.00)
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9904133.7	23 February 1999 (23.02.99)	GB																																					
9904404.2	25 February 1999 (25.02.99)	GB																																					
(54) Title: NEISSERIA MENINGITIDIS ANTIGENIC POLYPEPTIDES, CORRESPONDING POLYNUCLEOTIDES AND PROTE- CTIVE ANTIBODIES (57) Abstract <p>The invention provides Neisseria meningitidis BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 and BASB071 polypeptides, and polynucleotides encoding BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 and BASB071 polypeptides, and methods for producing such polypeptides by recombinant techniques. Also provided are diagnostic, prophylactic and therapeutic uses thereof.</p>																																							

Docket No.: BM45348

PCT/EP00/00135

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

NOVEL COMPOUNDS

the specification of which (check one)

- ☐ is attached hereto.
☒ was filed on 10 January 2000 as Serial No. PCT/EP00/00135
 and was amended on (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			
Number	Country	Filing Date	Priority Claimed
9900952.4	Great Britain	15 January 1999	Yes
9900838.5	Great Britain	15 January 1999	Yes
9901945.7	Great Britain	28 January 1999	Yes
9901948.1	Great Britain	28 January 1999	Yes
9902078.6	Great Britain	29 January 1999	Yes
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9902936.5	Great Britain	10 February 1999	Yes
9903978.6	Great Britain	20 February 1999	Yes
9904133.7	Great Britain	23 February 1999	Yes
9904404.2	Great Britain	25 February 1999	Yes

I hereby claim the benefit under Title 35, United States Code, Section 119(e) of any United States provisional application(s) listed below.

Application Number	Filing Date
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I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

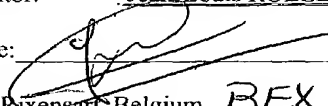
Serial No.	Filing Date	Status
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I hereby appoint the practitioners associated with the Customer Number provided below to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith, and direct that all correspondence be addressed to that Customer Number:

Customer Number 25,308

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1-00 Full Name of Inventor: Jean-Louis RUELLE

Inventor's Signature: 

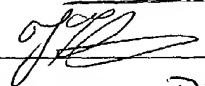
Date: 31 January 2002

Residence: Rixensart, Belgium BEX

Citizenship: Belgian

Post Office Address: SmithKline Beecham Corporation
Corporate Intellectual Property - UW2220
P.O. Box 1539
King of Prussia, Pennsylvania 19406-0939

2-00 Full Name of Inventor: Joelle THONNARD

Inventor's Signature: 

Date: 31.01.02

Residence: Rixensart, Belgium BEX

Citizenship: Belgian

Post Office Address: SmithKline Beecham Corporation
Corporate Intellectual Property - UW2220
P.O. Box 1539
King of Prussia, Pennsylvania 19406-0939

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SEQUENCE LISTING

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PCT/EP00/00135

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<210> 14
<211> 391
<212> PRT
<213> *Neisseria meningitidis*

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35 40 45
Glu Val Glu Leu Ile Asp Val Asn His Thr Val Ala Gln Leu Leu Tyr
50 55 60
Lys Ala Gln Ile Asn Gln Ser Phe Thr Gln Phe Gly Asp Gly Tyr Ala
65 70 75 80
Ser Ala Gly Thr Leu Asn Ile Gly Asp Val Leu Asp Ile Met Ile Trp
85 90 95
Glu Ala Pro Pro Ala Val Leu Phe Gly Gly Leu Ser Ser Met Gly

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100 105 110
 Ser Gly Ser Ala His Gln Thr Lys Leu Pro Glu Gln Leu Val Thr Ala
 115 120 125
 Arg Gly Thr Val Ser Val Pro Phe Val Gly Asp Ile Ser Val Val Gly
 130 135 140
 Lys Thr Pro Gly Gln Val Gln Glu Ile Ile Lys Gly Arg Leu Lys Lys
 145 150 155 160
 Met Ala Asn Gln Pro Gln Val Met Val Arg Leu Val Gln Asn Asn Ala
 165 170 175
 Ala Asn Val Ser Val Ile Arg Ala Gly Asn Ser Val Arg Met Pro Leu
 180 185 190
 Thr Ala Ala Gly Glu Arg Val Leu Asp Ala Val Ala Ala Val Gly Gly
 195 200 205
 Ser Thr Ala Asn Val Gln Asp Thr Asn Val Gln Leu Thr Arg Gly Asn
 210 215 220
 Val Val Arg Thr Val Ala Leu Glu Asp Leu Val Ala Asn Pro Arg Gln
 225 230 235 240
 Asn Ile Leu Leu Arg Arg Gly Asp Val Val Thr Met Ile Thr Asn Pro
 245 250 255
 Tyr Thr Phe Thr Ser Met Gly Ala Val Gly Arg Thr Gln Glu Ile Gly
 260 265 270
 Phe Ser Ala Arg Gly Leu Ser Leu Ser Glu Ala Ile Gly Arg Met Gly
 275 280 285
 Gly Leu Gln Asp Arg Arg Ser Asp Ala Arg Gly Val Phe Val Phe Arg
 290 295 300
 Tyr Thr Pro Leu Val Glu Leu Pro Ala Glu Arg Gln Asp Lys Trp Ile
 305 310 315 320
 Ala Gln Gly Tyr Gly Ser Glu Ala Glu Ile Pro Thr Val Tyr Arg Val
 325 330 335
 Asn Met Ala Asp Ala His Ser Leu Phe Ser Met Gln Arg Phe Pro Val
 340 345 350
 Lys Asn Lys Asp Val Leu Tyr Val Ser Asn Ala Pro Leu Ala Glu Val
 355 360 365
 Gln Lys Phe Leu Ser Phe Val Phe Ser Pro Val Thr Ser Gly Ala Asn
 370 375 380
 Ser Ile Asn Asn Leu Thr Asn
 385 390

<210> 15
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 <212> DNA
 <213> *Neisseria meningitidis*

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ggccccgatg tggatttgat gaacgcgatg gcgaaggcgg gcaatttta aatcgaattc 240
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atgtcggggc taaccattac cgacgaccgc aaacagtcta tggacttcag cgacccttat 360
tttgaaatca cccaagtcgt cctcgttccg aaaggcaaaa aaatatcttc ttccgaagat 420
ttgaaaaaca tgaacaaagt cggcgtggta accggctaca cgggcgattt ctccgtatcc 480
aaactcttgg gcaacgacaa cccgaaaatc gcgcgctttg aaaacgttcc cctgattatc 540
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tatgtgaaaa acaatccgac caaagggatg gacttcgtta ccctgcccg cttcaccacc 660
gaacactacg gcatcgcggt acgcaaaggc gacgaagcaa ccgtcaaaaat gctgaacgat 720
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<210> 16

<211> 268

<212> PRT

<213> *Neisseria meningitidis*

<400> 16

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      20             25             30
Ala Asn Pro Asp Lys Val Tyr Arg Val Ala Ser Asn Ala Glu Phe Ala
      35             40             45
Pro Phe Glu Ser Leu Asp Ser Lys Gly Asn Val Glu Gly Phe Asp Val
      50             55             60
Asp Leu Met Asn Ala Met Ala Lys Ala Gly Asn Phe Lys Ile Glu Phe
      65             70             75             80
Lys His Gln Pro Trp Asp Ser Leu Phe Pro Ala Leu Asn Asn Gly Asp
      85             90             95
Ala Asp Val Val Met Ser Gly Val Thr Ile Thr Asp Asp Arg Lys Gln
      100            105            110
Ser Met Asp Phe Ser Asp Pro Tyr Phe Glu Ile Thr Gln Val Val Leu
      115            120            125
Val Pro Lys Gly Lys Lys Ile Ser Ser Ser Glu Asp Leu Lys Asn Met
      130            135            140
Asn Lys Val Gly Val Val Thr Gly Tyr Thr Gly Asp Phe Ser Val Ser
      145            150            155            160
Lys Leu Leu Gly Asn Asp Asn Pro Lys Ile Ala Arg Phe Glu Asn Val
      165            170            175
Pro Leu Ile Ile Lys Glu Leu Glu Asn Gly Gly Leu Asp Ser Val Val
      180            185            190
Ser Asp Ser Ala Val Ile Ala Asn Tyr Val Lys Asn Asn Pro Thr Lys
      195            200            205
Gly Met Asp Phe Val Thr Leu Pro Asp Phe Thr Thr Glu His Tyr Gly
      210            215            220
Ile Ala Val Arg Lys Gly Asp Glu Ala Thr Val Lys Met Leu Asn Asp

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225                230                235                240
Ala Leu Lys Lys Val Arg Glu Ser Gly Glu Tyr Asp Lys Ile Tyr Ala
                245                250                255
Lys Tyr Phe Ala Lys Glu Asp Gly Gln Ala Ala Lys
                260                265

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